THE BIOSYSTEMATICS OF *LUDWIGIA* SECT. *MICROCARPIUM* (ONAGRACEAE)[1]

Ching-I Peng[2]

ABSTRACT

Ludwigia *sect.* Microcarpium *is a polyploid complex of 14 species distributed primarily in the southeastern United States. Several of the species are variable and taxonomically difficult; the boundaries of many are blurred by intermediate forms. Relationships among the species are often reticulate and in some cases difficult to specify precisely. Data from the study of meiosis in artificial and natural hybrids were used to analyze relationships among the species. Among diploid* (n = 8) *species,* L. linearis *and* L. linifolia *are similar in morphology but differ chromosomally by one reciprocal translocation.* F_1 *hybrids between them are vigorous, have 47–48% stainable pollen, and produce moderate quantities of viable seeds. Another diploid,* L. microcarpa, *is morphologically and genetically distinct from* L. linearis *and* L. linifolia. *Hybrids between* L. microcarpa *and either other diploid are vigorous but produce only 6% stainable pollen and no viable seeds. They formed mostly univalents at meiotic metaphase I, with up to three sometimes heteromorphic bivalents. The fourth diploid,* L. stricta, *has not been studied biosystematically. The eight morphologically distinct tetraploid* (n = 16) *taxa can be crossed in any combination, producing fertile offspring with complete association of their 16 bivalents. No extant diploids are believed to have been involved directly in forming the tetraploids of sect.* Microcarpium. Ludwigia alata, *a hexaploid* (n = 24), *is morphologically similar to the tetraploids, and chromosome pairing in experimental hybrids suggests that it originated after hybridization between a tetraploid and* L. microcarpa *or populations ancestral to that species. Two chromosome numbers are present in the* L. curtissii *complex (*L. simpsonii, n = 24; L. curtissii, n = 32). *The hexaploid complement of* L. simpsonii *appears to include three different diploid genomes, one of which is identical with that of* L. microcarpa. *Present data are not sufficient to determine if the diploid* L. linearis–L. linifolia *lineage was involved in parentage of the hexaploid* L. simpsonii, *but morphological evidence suggests it was not. It is highly probable, however, that the octoploid* L. curtissii *was derived after hybridization between the diploid* L. linearis–L. linifolia *lineage and the hexaploid* L. simpsonii.

Ludwigia contains some 82 species, which are classified into 23 sections (Raven, 1963; Ramamoorthy, 1979; Ramamoorthy & Zardini, 1987). It is the only member of the monotypic tribe Jussiaeeae and is the fourth largest genus in Onagraceae (after *Epilobium*, *Oenothera*, and *Fuchsia*). It has existed since at least the Eocene (Eyde & Morgan, 1973), and increasing evidence suggests it represents the earliest surviving evolutionary offshoot of the family (review in Ramamoorthy & Zardini, 1987; Eyde, 1981), or, in cladistic terms, the "sister group" of all other genera of the family. Study of this genus is therefore critical to understanding the overall evolutionary pattern in Onagraceae, a subject to which Peter H. Raven and his associates have devoted much time and energy over the past 30 years.

Thirteen species of *Ludwigia* are restricted to the Old World, 59 to the New World, and 10 are common to both hemispheres (Ramamoorthy,

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[2] *Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan 11529, Republic of China.*

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1980). Most sections are tropical and subtropical, but *Ludwigia* also has several well-developed temperate offshoots, particularly in North America (Raven & Tai, 1979).

Most native North American *Ludwigia* species have four sepals, four stamens, pluriseriate and free seeds with narrow raphes, and a herbaceous habit (Raven, 1963). With a few exceptions they are confined to the Atlantic and Gulf coastal plains of the United States. The 23 haplostemonous species represented here are placed in three sections: sect. *Ludwigia*, with 4 species (Munz, 1944, 1965; Raven, 1963); sect. *Dantia*, with 5 species (Schmidt, 1967); and sect. *Microcarpium*, a diverse group of 14 species (Table 1), which constitutes the subject of this study.

Section *Microcarpium* was selected for the present study because it represents a diverse polyploid complex (Raven & Tai, 1979) in which abundant natural hybridization occurs and in which the relationships among species are not clear. In a comparative study of the reproductive structures in *Ludwigia*, Eyde (1978) considered sect. *Microcarpium* as "remarkably diverse," at least with respect to surface-cell orientation of the seeds. The fact that several polyploid species were reported to have two different chromosome numbers (Raven & Tai, 1979) may reflect either difficulties in identification or their diverse genetic backgrounds. Duke (1955) noted the varying degree of intergradation exhibited between North Carolina *Ludwigia* species, and he suspected one population to be of hybrid origin between two species of sect. *Microcarpium*. A critical study of herbarium material has revealed many specimens exhibiting various intermediate morphological characteristics. Two such natural hybrids, *L. lanceolata* × *L. pilosa* and *L. suffruticosa* × *L. pilosa*, have even been given specific and varietal names (*L. simulata* Small and *L. suffruticosa* Walter var. *pubens* Torrey & A. Gray, respectively). Conclusive evidence of intergradation among species, however, can only be obtained through experimental hybridization.

Schmidt's (1967) work in establishing the phylogenetic relationships among species belonging to *Ludwigia* sect. *Dantia* has proved helpful in the study of sect. *Microcarpium*. Both of these sections were grouped, along with the monotypic East Asian section *Miquelia*, in the "*Microcarpium* complex" on the basis of reproductive structures and overall evolutionary patterns in *Ludwigia* (Eyde, 1981). Schmidt (1967) demonstrated through artificial hybridization and cytological observations of hybrid microsporocytes in sect. *Dantia* that hybrids between plants of different ploidy levels consistently exhibited typical "Drosera-type" chromosome pairing; i.e., homologous genomes in the F_1 generations always formed bivalents and nonhomologous genomes remained unpaired.

Schmidt (1967) also studied two naturally occurring intersectional hybrids. Meiotic observations of the hybrid between *L. palustris* ($n = 8$; sect. *Dantia*) and *L. glandulosa* ($n = 16$; sect. *Microcarpium*) revealed very little association between chromosomes, with 0–3 weakly joined bivalents. The other natural hybrid examined, involving *L. simpsonii* ($n = 24$, cited as *L. curtissii* by Schmidt; sect. *Microcarpium*) and *L. repens* ($n = 24$; sect. *Dantia*), yielded 48 unpaired chromosomes at meiosis. These observations suggest that in the related sect. *Microcarpium*, "Drosera-type" chromosome pairing also occurs. This characteristic is helpful in studying genetic relationships among species in such a polyploid complex.

The purpose of the present study was to determine the evolutionary relationships of the taxa comprising sect. *Microcarpium*. Toward this end I have considered extensive cytological evidence from my experimental hybridizations. This has been supplemented with data on morphology, pollination biology, crossing relationships, geographical distribution, and field observations of morphological variation and habitat preference. In addition, more than 7,000 herbarium specimens have been examined.

Materials and Methods

Specimens were collected from wild populations and were propagated from seeds or clonal transplants in the experimental greenhouse at the Missouri Botanical Garden (with the exception of some strains that were obtained as seeds from the Kew Seed Bank). Members of sect. *Microcarpium* can be cloned easily from their vegetative parts and cultivated in the greenhouse by standard procedures. Parental seeds usually germinated readily one or two weeks after they were sown. However, it took one or two months or even longer for some of the hybrid seeds to germinate. Plants of sect. *Microcarpium* normally flower the first year.

In an insect-free greenhouse, several flowers of each parental strain were (1) artificially self-pollinated to test for self-compatibility, (2) left alone to test for self-pollination, and (3) emasculated and left alone to test for apomixis. Plants of all species studied were found to be self-compatible and non-apomictic. Except for a few species, in which selfing is prevented physically, members of sect. *Microcarpium* are capable of mechanical self-pollination.

All experimental crosses were therefore made by first emasculating the ovulate parent before self-pollination could occur, and then applying pollen from the pollen parent to its stigmas. Generally, several different parental strains were used in each cross; in a given trial all pollen parents were from the same population. The seeds resulting from successful hybridizations were sown early the following spring. Usually six seedlings of each artificial hybrid were randomly selected and transplanted into five-inch pots. A few of the F_2 families were also grown to maturity. About five to six months were required to produce a flowering hybrid after germination.

Flower buds to be examined for meiotic behavior were fixed in a 3:1 mixture of 95% ethanol and glacial acetic acid and stored in the refrigerator. Prior to staining, the buds were hydrolyzed for 5–8 minutes at 60°C using a 1:1 mixture of concentrated HCl and 95% ethanol. They were then squashed in FLP orcein (Jackson, 1973). Somatic chromosome counts for some of the parental strains were obtained from actively growing root tips pretreated for three to four hours in 8-hydroxyquinoline, then fixed as above for at least ten minutes. The root tips were then hydrolyzed in 1 N HCl for 8–10 minutes at 60°C and squashed in the FLP orcein. Semipermanent slides were prepared and preserved in the freezer. Cytological observations were made using a Zeiss Universal Large Research Microscope. All analyzable chromosome configurations (mostly diakinesis or metaphase I) were documented with camera lucida drawings or photomicrographs using Kodak Panatomic-X film. Negatives and drawings are deposited at the Institute of Botany, Academia Sinica, Taipei.

Fertility of greenhouse parental individuals, experimental hybrids, and suspected naturally occurring hybrids was estimated by determining the percentage of stainable pollen using the malachite green-acid fuchsin-orange G stain of Alexander (1969), which stains pollen walls green and cytoplasm red. Pollen grains with uniformly red cytoplasm were scored as fertile; partially stained or unstained grains were considered sterile. In many hybrids, however, and especially those of heteroploid crosses, the stainable pollen grains differ substantially in size, and some probably are not functional, as was suggested by Uhl (1976). At least 200 tetrads (when pollen shed as tetrads) or 400 single grains (when pollen shed singly) per plant were scored.

Seeds of members of sect. *Microcarpium* are so small (ca. 0.4–0.7 mm long) that studying the shapes and orientations of their surface cells under a dissecting microscope is very difficult. However, they are transparent enough to be examined under a light microscope. Photographs of seeds were taken with strong back illumination using Kodak Panatomic-X film. Polarized light was sometimes used.

Herbarium specimens prepared from all experimental plants, both parental and hybrid (with the exception of the very small individuals such as those which did not develop much further than the cotyledonous stage), are deposited at MO. Experimental hybrids are designated by a formula consisting of the acronym (Tables 3–15; Figs. 5, 21) for the two parents, connected by the multiplication sign (×), with the ovulate parent listed first.

Diagnostic Features

Discussion of the morphological features is limited to those useful in delimiting members of sect. *Microcarpium* and in recognizing hybrids. A more complete discussion of morphological variability in this group can be found in Peng (in press). Characters that are functionally related to the pollination biology will be examined in more detail later in this paper. Some relevant diagnostic characters are summarized in Table 1.

HABIT

Plants of this section are all erect perennial herbs about 15–100 cm tall. They produce sprawling, leafy stolons along the surface of the ground in winter, although some species may also send out stolons in the summer while they are still flowering. In *Ludwigia suffruticosa*, however, underground rhizomes with scalelike leaves are also produced.

FLOWERS

The flowers have four sepals, four stamens, and a four-loculed ovary. Only *L. linearis*, *L. linifolia*, and *L. stricta*, all diploid, consistently have four yellow petals. The only other diploid species, *L. microcarpa*, and tetraploid, hexaploid, and octaploid species are apetalous. Vestigial petals are, however, occasionally present in normally apetalous species, especially *L. curtissii*. Loss of petals apparently represents the derived status, which is reflected in the fact that all polyploid species lack petals. Absence of petals does not in itself indicate autogamy, however, since nectary discs on top of the ovaries of all species produce various amounts of nectar that is fed on by insects. Furthermore, in the apetalous species—*L. alata*, *L. suffruticosa*, *L. pilosa*, and *L. sphaerocarpa*—the sepals are either cream-colored or yellow and are quite showy.

One aspect of the floral morphology of particular

TABLE 1. *Some characters of* Ludwigia *sect.* Microcarpium.

Taxon	Chromosome Number	Petals Present (+) or Absent (−)	Pollen Shed Singly (S) or as Tetrads (T)	Seed Surface Cell Shape and Orientation[a]
L. alata Elliott	$n = 24$	−	S	T
L. curtissii Chapman	$n = 32$	−[b]	S	T
L. glandulosa Walter				
subsp. *glandulosa*	$n = 16$	−	T	P
subsp. *brachycarpa* (Torrey & A. Gray) Peng	$n = 16$	−	T	T
L. lanceolata Elliott	$n = 16$	−	T	I
L. linearis Walter	$n = 8$	+	T	P or T[c]
L. linifolia Poiret	$n = 8$	+	T	I
L. microcarpa Michaux	$n = 8$	−	S	T
L. pilosa Walter	$n = 16$	−	T	I
L. polycarpa Short & Peter	$n = 16$	−	T	P
L. ravenii Peng	$n = 16$	−	T	T
L. simpsonii Chapman	$n = 24$	−	S	T
L. sphaerocarpa Elliott	$n = 16$	−	T	P and T[d]
L. stricta (Wright ex Griseb.) Wright	$n = 8$	+	T	I
L. suffruticosa Walter	$n = 16$	−	S	I

[a] Letter codes I, P, T indicate seeds with surface cells more or less isodiametric, in columns parallel elongate to the seed length, and transversely elongate to the seed length, respectively.

[b] Flowers with 1–3 (sometimes 4) vestigial petals sometimes observed.

[c] Seed surface cells elongate parallel to the seed length are predominantly exhibited by the subglabrous populations, whereas cells elongate transversely to the seed length are prevalent in more strigillose populations.

[d] Seed surface comprised of a mixture of cells both parallel and transversely elongate to the seed length. Sometimes the orientation of these columnar cells is irregular.

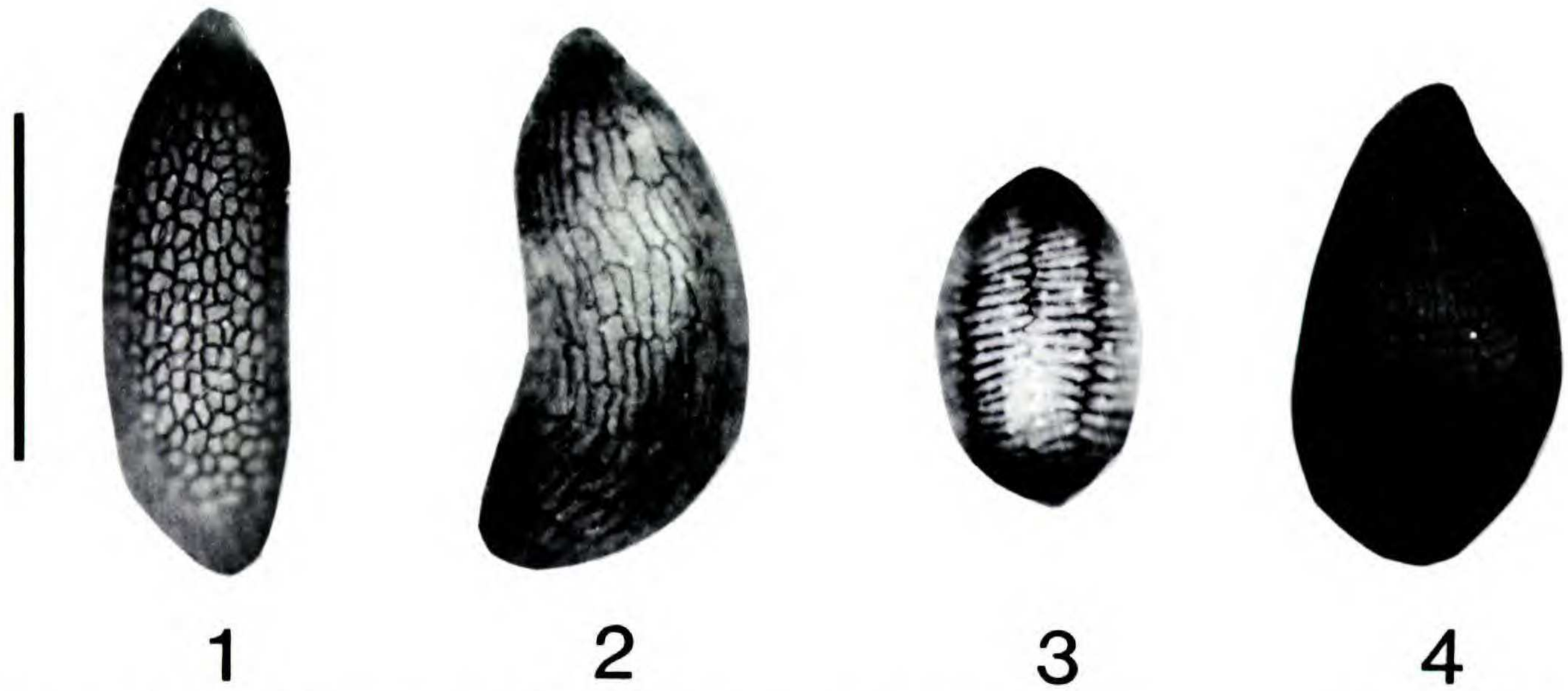
interest in *L. linearis* is the presence in the anthers of transverse septa composed of tapetum and parenchyma which divide the sporogenous tissue into packets (Eyde, 1977; Tobe & Raven, 1986). This character is shared only by an unrelated South American species, *L. latifolia* (Benth.) Hara, and five other genera of Onagraceae.

POLLEN

Pollen morphology of Onagraceae has been studied intensively by Ting (1966), Brown (1967), Skvarla et al. (1975, 1976, 1978), and Praglowski et al. (1983). Unique palynological features of the family include: protruding papillose apertures; mechanisms of tetrad and polyad cohesion; the fine structure of the exine; and viscin threads, which are extensions of the exine that tend to bind the grains together in masses. Pollen of sect. *Microcarpium* is quite uniform, being characterized by isopolar grains frequently with prominent colpal extensions and with a psilate exine (Praglowski et al., 1983). Most species shed pollen in tetrads, although in *L. alata*, *L. curtissii*, *L. microcarpa*, *L. simpsonii*, and *L. suffruticosa* grains are shed singly (in monads), a characterisic found sporadically in other species of *Ludwigia* (Praglowski et al., 1983). Pollen shed as monads is thought to be the ancestral condition in *Ludwigia* (Praglowski et al., 1983), but in view of the relationships suggested by Eyde (1977, 1978, 1981), the monad pollen in sect. *Microcarpium* was probably derived secondarily from tetrad pollen. In any event, this character, along with the seed-surface cell pattern, is useful in distinguishing closely related species like *L. alata* ($n = 24$; monads) and *L. lanceolata* ($n = 16$; tetrads), which have different chromosome numbers but are otherwise difficult to distinguish. It is also highly helpful in detecting natural hybrids when their suspected parents differ in this character.

CAPSULES

Fruit anatomy of *Ludwigia* has been studied by Eyde (1978), who reported that the fruit wall in *L. alata* is thickest in the placental radii, contrasting markedly with the fruit wall in sect. *Dantia* and in most other species of sect. *Microcarpium*. The shape, size, and vestiture of capsules are very



FIGURES 1–4. Photographs of seeds of some members of *Ludwigia* sect. *Microcarpium*.—1. *L. lanceolata*, Florida: Highlands Co., *Peng 4183* (MO).—2. *L. glandulosa* subsp. *glandulosa*, Florida: Santa Rosa Co., *Dille 412* (MO).—3. *L. alata*, Florida: Wakulla Co., *Morar 11* (FSU).—4. *L. sphaerocarpa*, South Carolina: Jasper Co., *Dille 348* (MO). Scale bar = 0.4 mm.

diverse within sect. *Microcarpium*. Capsule shape ranges from obpyramidal to subcylindrical, oblong-obovate, turbinate, or subglobose, and length ranges from 1 to 12 mm. In *L. alata* and *L. lanceolata*, the capsules are narrowly to markedly four-winged. The surface vestiture ranges from glabrous to strigillose or hirtellous. These characters are of paramount importance in distinguishing the species and detecting hybrids. Furthermore, the lengths of persistent bracteoles at or near the capsule base is often a diagnostic character in species delimitation and hybrid recognition.

SEEDS

The seeds are small, 0.4–0.7 mm long, and are cylindric, ellipsoid, reniform, or ovoid in shape. Their surface cells are diverse in shape and orientation (Eyde, 1978). They are either more or less isodiametric (Fig. 1) or are in parallel columns that are predominantly either elongate parallel (Fig. 2) or transversely elongate (Fig. 3) to the seed length, with minor variation on the two ends and areas near the raphe (Table 1). Like capsule morphology, seed-surface pattern provides excellent diagnostic characters for identifying the species and detecting natural hybrids.

In *L. sphaerocarpa* the seed-surface cells (Fig. 4) are less regularly oriented than in the other species. They are arranged in columns both transversely elongate and parallel to the seed length, with the former alignment often dominant in the central part of the seed. In this species, it is also not uncommon to have some seeds with variously oriented cells, a pattern that supports the suggestion of a hybrid origin for *L. sphaerocarpa* (see below).

REPRODUCTIVE BIOLOGY

Raven (1979) thoroughly reviewed reproductive biology of Onagraceae. Two-thirds (56 of 82) of all species in *Ludwigia* modally self-pollinate, and of the 26 that modally outcross, most accomplish that by separation of the stigma and anthers (Raven, 1979; Ramamoorthy & Zardini, 1987). There are no known instances in *Ludwigia* of protandry, protogyny, or male sterility, such as are found in other genera of Onagraceae (Raven, 1979). In nine species of *Ludwigia*, however, outcrossing is reinforced by genetic self-incompatibility, which occurs in about a quarter of all outcrossing species in the family (Raven, 1979).

All members of sect. *Microcarpium* are genetically self-compatible perennials that are mostly facultatively autogamous. Shortly after the flowers open in the morning, the anthers dehisce and the stigma becomes receptive. The anthers spread and are held away from the stigma shortly after anthesis, but in most species, petalous or apetalous, the anthers arch over a few hours later and attach firmly to the sticky stigma, thus effecting self-pollination.

Self-pollination, as well as cross-pollination, however, can also be achieved by insect vectors without having the anthers attached to the stigma mechanically. Bumble bees, honeybees, wasps, moths, and ants were observed visiting populations of *L.*

pilosa in the field (Peng, 1984). Raven (pers. comm.) also observed numerous wasps attracted by flowers of *L. sphaerocarpa*. The presence of plentiful confirmed natural hybrids (see below) suggests substantial cross-pollination by insects in the field.

Cytology

The first cytological study of a member of sect. *Microcarpium* was made by Gregory & Klein (1960) who, in the course of investigating the meiotic chromosomes of several onagraceous genera, recorded five counts for four species (cited as five species). These authors were the first to document polyploidy in the genus. One diploid population of *L. linifolia* that they studied was subsequently examined mitotically by Kurabayashi et al. (1962), who called attention to the fact that the chromosomes of *Ludwigia* and those of tribe Epilobieae are the smallest in Onagraceae, and also that they may differ conspicuously in size within a single genome. The proximal ends of the chromosome arms are heavily pycnotic and appear even in interphase nuclei as very distinct and definite chromocenters.

Based on a review of the literature and the study of 302 individuals from 282 naturally occurring populations from throughout the range of the genus, Raven & Tai (1979) presented a comprehensive chromosome number report for 38 of the 45 species of *Ludwigia* exclusive of sect. *Myrtocarpus* sensu lato. The basic chromosome number for the genus was established as $x = 8$ with no aneuploidy but extensive polyploidy. In my description of a new species of sect. *Microcarpium*, *L. ravenii* (Peng, 1984), I reported $2n = 32$ for this species, which has been misidentified as *L. pilosa* in the past. *Ludwigia stricta*, a Cuban endemic, is here reported as a diploid, with $n = 8$. Through these efforts, chromosome counts are now available for all taxa recognized in sect. *Microcarpium*. Section *Microcarpium* is shown to be a diverse polyploid complex, with four diploids, eight tetraploids, two hexaploids, and an octoploid. *Ludwigia alata*, *L. curtisii*, and *L. suffruticosa*, reported as having more than one chromosome number (Raven & Tai, 1979), will be discussed below.

In the present paper, I am reporting 78 more counts representing 75 populations of 14 species and one additional subspecies in sect. *Microcarpium* (Table 2). The chromosome number of *L. stricta* is here reported for the first time. These, along with 69 previously reported counts by Raven & Tai (1979), constitute our present knowledge of cytology of sect. *Microcarpium*. Because several taxonomic changes have been made, including a new combination (Peng, 1986), and because a new species, *L. ravenii*, has recently been recognized (Peng, 1984), I have checked the identification of voucher specimens cited by Raven & Tai (1979) and included these previously published counts in Table 2, using the currently accepted names. All counts are gametic except those indicated by "$2n =$ ". Original counts reported by Raven & Tai (1979) are indicated by asterisks; those reported by others are accompanied by references.

Raven & Tai (1979), while reporting two chromosome numbers for *L. alata*, stated that "Chromosome counts are now available for all taxa except . . . *L. stricta* . . . and, if it is distinct from *L. alata*, *L. lanceolata* Ell." Indeed, the last two species are similar in many aspects, especially in sharing obpyramidal capsules with winged corners, which is the key character in recognizing this species pair. However, upon examination of microscopic characters, such as seed surface architecture and the way pollen grains are shed, characters which previously have not been used by monographers (Munz, 1944, 1965), it has become clear that the two are distinct species. *Ludwigia alata* consistently sheds pollen singly (Praglowski et al., 1983) and has seed-surface cells in parallel columns transversely elongate to the seed length (Fig. 3), whereas *L. lanceolata* sheds pollen as tetrads (Praglowski et al., 1983) and invariably has nearly isodiametric seed-surface cells (Fig. 1). Their seeds differ in size and shape as well. Furthermore, these differences are correlated with chromosome number. Based on the 15 counts available (Table 2), *L. alata* is hexaploid with $n = 24$, whereas *L. lanceolata* is tetraploid with $n = 16$. The only exception is a specimen collected from Collier Co., Florida (*Raven 18672*), which Raven & Tai (1979) correctly identified as *L. alata* and reported to have $n = 16$. In order to verify this report, the same population was sampled again in 1980 (*Peng 4242*). Plants from this population yielded a count of $n = 24$. It seems likely, therefore, that the reported count of $n = 16$ resulted from confusion or interchange of samples. The questionable count has therefore been omitted from Table 2.

For *L. suffruticosa* there are 11 chromosome counts available at present, ten with $n = 16$, and one with $n = 24$ for a collection (*Raven 18651*) from Hillsborough Co., Florida (Raven & Tai, 1979). Four other populational counts obtained from the same county consistently show $n = 16$. It is possible that plants with $n = 24$ have arisen

TABLE 2. *Chromosome numbers in* Ludwigia *sect.* Microcarpium, *with voucher information. Voucher specimens for original counts are at the Missouri Botanical Garden (MO); those of earlier reports, identified by an asterisk, are indicated in Raven & Tai (1979).*

Ludwigia alata Elliott ($n = 24$)

U.S.A. FLORIDA: Collier Co., *Peng 4242* ($2n = 48$), *Peng 4267* ($2n = 48$); Franklin Co., *Godfrey 70575*, *Peng 4344*; Levy Co., *Dille 392*; Martin Co., *Peng 4203* ($n = 24$; $2n = 48$); Wakulla Co., *Raven 18608**. GEORGIA: Charlton Co., nr. Cravens Hammock, *Raven in 1974** ($2n = 48$). SOUTH CAROLINA: Horrey Co., *Raven 18719**.

Ludwigia curtissii Chapman ($n = 32$)

BAHAMA ISLANDS. Grand Bahama Island, *Correll & Popenoe 51315.*

U.S.A. FLORIDA: Collier Co., *Peng 4231, 4276, 4283*; Dade Co., *Godfrey 63396** ($2n = 64$); Franklin Co., *Godfrey 71148*; Hendry Co., *Peng 4285, 4287*; Hillsborough Co., *Dille 435*; Martin Co., *Peng 4199*; Monroe Co., *Godfrey 63519** ($2n = 64$); Palm Beach Co., *Popenoe 1962*; Sarasota Co., *Raven 18662** ($2n = 64$).

Ludwigia glandulosa Walter subsp. *glandulosa* ($n = 16$)

U.S.A. ALABAMA: Macon Co., *Raven 18562**. ARKANSAS: *Demaree 46645** (*Raven 65-42*, $2n = 32$). FLORIDA: Jefferson Co., *Raven 18617**; Leon Co., *Godfrey* (Gregory & Klein, 1960); Madison Co., *Raven 18628**; Santa Rosa Co., *Dille 412*. GEORGIA: Emanuel Co., *Peng 4013* ($2n = 32$). LOUISIANA: St. Tammany Parish, *Raven 18576**, *18577**. MISSISSIPPI: Jones Co., *Raven 18569**. NORTH CAROLINA: Columbus Co., *Broome 865, 897* TEXAS: Liberty Co., *Raven 19427**.

Ludwigia glandulosa subsp. *brachycarpa* (Torrey & A. Gray) Peng ($n = 16$)

U.S.A. LOUISIANA: Cameron Parish, *Peng 4367*. TEXAS: Fort Bend Co., *Raven 19398** (as *L. glandulosa*), *Raven 19405** (as *L. glandulosa*).

Ludwigia lanceolata Elliott ($n = 16$)

U.S.A. FLORIDA: Highlands Co., *Dille 370* ($2n = 32$), *Peng 4183, 4193*, *Raven 18681** (as *L. alata*), *Raven 18684** (as *L. alata*), *Raven 19727** (as *Raven 17927*, typographic error, $2n = 32$, as *L. alata*).

Ludwigia linearis Walter ($n = 8$)

U.S.A. ALABAMA: Baldwin Co., *Raven 18590**. ARKANSAS: *Demaree 46898** (*Raven 65-43*, $2n = 16$). FLORIDA: Madison Co., *Raven 18627**. GEORGIA: Emanuel Co., *Peng 4023* ($2n = 16$). LOUISIANA: St. Tammany Parish, *Dille 420*, *Raven 18579**. MISSISSIPPI: Jackson Co., *Raven 18585**. NORTH CAROLINA: Cumberland Co., *Lloyd 1026**; Johnston Co., *Lloyd 1121**. SOUTH CAROLINA: Horry Co., *Raven 18721**; Jasper Co., *Dille 350*, *Peng 3935* ($2n = 16$).

Ludwigia linifolia Poiret ($n = 8$)

MEXICO. TABASCO:Municipio Huimanguillo, *Cowan 2632* (also $2n = 16$), *Cowan 3111.*

U.S.A. FLORIDA: Franklin Co., *Peng 4343*; Hillsborough Co., *Dille 427*; Okaloosa Co., *Raven 18593**; Wakulla Co., *Godfrey 77091* ($2n = 16$). MISSISSIPPI: Hancock Co., *Raven 18581**; Jackson Co., *Demaree 37879* ($n = 8$, Gregory & Klein, 1960; $2n = 16$, Kurabayashi et al., 1962).

TABLE 2. *Continued.*

Ludwigia microcarpa Michaux ($n = 8$)

U.S.A. FLORIDA: Charlotte Co., *Peng 4294*; Clay Co., *Dille 359*, *Raven 18692**; Franklin Co., *Peng 4348*; Hillsborough Co., *Raven 18641**; Jackson Co., *Godfrey 77093*; Wakulla Co., *Raven 18601**, *18610**. NORTH CAROLINA: Jones Co., *Peng 3800.*

Ludwigia pilosa Walter ($n = 16$)

U.S.A. FLORIDA: Franklin Co., *Peng 4345*; Leon Co., *Kral in 1963** (*Raven 65-44*, $2n = 32$); Madison Co., *Raven 18625**; Walton Co., *Raven 18594**. GEORGIA: Camden Co., *Raven 18701**; Emanuel Co., *Peng 4025* ($2n = 32$). MISSISSIPPI: Hancock Co., *Dille 419* ($2n = 32$), *Raven 18580**; Jackson Co., *Raven 18583**; Jones Co., *Raven 18568**. SOUTH CAROLINA: Colleton Co., *Raven 18717**; Horry Co., *Dille 342*; Jasper Co., *Raven 18712**.

Ludwigia polycarpa Short & Peter ($n = 16$)

U.S.A. MASSACHUSETTS: Middlesex Co., *Raven 16514**. MICHIGAN: Washtenaw Co., *Raven 16523**. MISSOURI: Franklin Co., *Dille 328* ($2n = 32$), *Dille 436* ($2n = 32$); Lincoln Co., *Dille 443* (also $2n = 32$).

Ludwigia ravenii Peng ($n = 16$)

U.S.A. FLORIDA: Clay Co., *Raven 18690** (as *Raven 19690*, typographic error; as *L. pilosa*). SOUTH CAROLINA: Berkeley Co., *Peng 4402* ($2n = 32$, Peng, 1984).

Ludwigia simpsonii Chapman ($n = 24$)

U.S.A. FLORIDA: Charlotte Co., *Peng 4293*; Collier Co., *Dille 378*, *Munz & Gregory 23476* (Gregory & Klein, 1960, as *L. curtissii*), *Peng 4232, 4234, 4246, 4248, 4254, 4261, 4262, 4268, 4271*; Hillsborough Co., *Raven 18649** (also $2n = 48$, as *L. curtissii*); Lee Co., *Peng 4289*; Martin Co., *Munz & Gregory 23481* (Gregory & Klein, 1960); Sarasota Co., *Dille 383*, *Peng 4313*, *Raven 18664** (as *Raven 18640*, typographic error).

Ludwigia sphaerocarpa Elliott ($n = 16$)

U.S.A. Without definite locality, *Monoson 55* (Gregory & Klein, 1960). FLORIDA: Franklin Co., *Dille 402*; Madison Co., *Raven 18626**, *18630**; Taylor Co., *Raven 18620**; Wakulla Co., *Dille 401*, *Peng 4339*. INDIANA: Starke Co., *Raven 16525**. MASSACHUSETTS: Plymouth Co., *Raven 16516**. SOUTH CAROLINA: Jasper Co., *Dille 348* ($2n = 32$).

Ludwiga stricta (Wright ex Griseb.) Wright ($n = 8$)[a]

CUBA. *A. Leiva s.n. in 1982* ($2n = 16$).

Ludwigia suffruticosa Walter ($n = 16$)

U.S.A. FLORIDA: Glades Co., *Raven 18678** ($2n = 32$); Hillsborough Co., *Dille 423, 424, 434*, *Peng 4327*; Lake Co., *Raven 18637*; Leon Co., *Dille 421*, *Raven 18595** (as *Raven 18585*, typographic error); Polk Co., *Lakela 24806** (*Raven 19704*, $2n = 32$); Taylor Co., *Raven 18619**.

[a] Chromosome number here determined for the first time.

directly from tetraploids by fusion of an unreduced gamete from one parent with a normal gamete from the other, as Raven & Tai (1979) postulated. Meiosis was normal (Raven & Tai, 1979), and pollen from the voucher specimen was fully viable

as judged by staining results. The count of $n = 24$ should be reconfirmed and investigated further if additional individuals are located; it is omitted from Table 2.

Ludwigia curtisii is another species Raven & Tai (1979) reported as having two chromosome numbers. They considered this species, along with *L. simpsonii* and *L. spathulifolia*, to comprise a single species complex, within which diagnostic characters such as capsule size and leaf shape (Munz, 1944, 1965) were not correlated with chromosome numbers. The present study, however, indicates that *L. simpsonii* is specifically distinct from *L. curtissii* and that *L. spathulifolia* should be treated as a variant of *L. curtissii* with slightly larger capsules.

In addition to being high polyploids (hexaploid and octoploid), plants of this complex are unique among species of sect. *Microcarpium* in having the capsules split along four longitudinal lines opposite the loculi at maturity (Peng & Tobe, 1987). Capsules from other species in sect. *Microcarpium* dehisce either by separation of the walls from the indurate nectary disc (Munz, 1944, 1965; Raven, 1963) or by irregular disintegratoin of the fruit wall (Peng & Tobe, 1987). Technical characters, such as the way pollen grains are shed and seed-surface cell shape and orientation, are not useful in distinguishing these taxa; they have both pollen grains shed singly and seed-surface cells in parallel columns transversely elongate to the seed length. They have turbinate or slightly broadly turbinate capsules 1.5–4.5 mm long and are extremely variable in cauline leaf shape, which ranges from obovate to spatulate-oblanceolate, narrowly oblanceolate, or sublinear. No consistent correlation was found between leaf shape and capsule size. When plants of distinct leaf shape and capsule size were collected and cultivated in an experimental greenhouse, the leaf shapes tended to converge and become spatulate-oblanceolate, but the capsule sizes remained constant.

Based on counts from 30 populations, including seven reported by Raven & Tai (1979), differences in chromosome number are indeed correlated with mature capsule size. All plants with $n = 24$ have mature capsules 1.5–2(–2.5) mm long, while all plants with $n = 32$ have mature capsules (2–)2.5–4(–4.5) mm long. The morphology of plants with $n = 24$ fits very closely the description of *L. simpsonii*, whereas the morphology of plants with $n = 32$ clearly corresponds to that of *L. curtissii* (including *L. spathulifolia*).

Compared with plants with $n = 32$, plants with $n = 24$ have generally diminutive floral parts: shorter bracteoles, sepals, stamens, and ovaries. Further, plants with $n = 24$ rarely exhibit vestigial petals, whereas those with $n = 32$ frequently have 1–3 caducous petals on at least some flowers. Vegetatively, plants with $n = 24$ are erect to ascending, or sometimes are prostrate and rooting at the nodes. They are pale green with slender stems often much branched from below or above. The leaves are sometimes quite small, tending to be opposite or subopposite at the lower nodes. By contrast, plants with $n = 32$ are usually dark green or sometimes purplish, have stouter erect stems branched above, and leaves usually alternate throughout.

Although *L. simpsonii* and *L. curtissii* frequently are sympatric, they are ecologically distinct and seldom actually intermix. Plants with $n = 24$ tend to grow along roadsides with other weeds in moist sandy soil. Plants with $n = 32$ grow far from roadsides in black muck and often in deep standing water, often mixed with tall grasses or sedges.

In view of this evidence, *L. simpsonii* is considered a hexaploid with $n = 24$, and *L. curtissii* (including *L. spathulifolia*) an octoploid with $n = 32$.

A collection from Clay Co., Florida (*Raven 18690*) was originally identified as *L. pilosa* and determined to be tetraploid with $n = 16$ (Raven & Tai, 1979). A more detailed morphological study reveals that this is best treated as *L. ravenii* (Peng, 1984). Nearly all previous collections of *L. ravenii* were initially identified as *L. pilosa* by their respective collectors: the two species are similar in being densely hirtellous, a distinct character not shared by any other member of sect. *Microcarpium*. *Ludwigia ravenii* may be distinguished readily from *L. pilosa* by having oblong-obovoid capsules and seed-surface cells predominantly in parallel columns transversely elongate to the seed length, and by having shorter sepals, filaments, and styles. Furthermore, unlike *L. pilosa*, which is nearly always hirtellous on the lower half of the style and between the lobes of the nectary disk, all 28 collections of *L. ravenii* are completely glabrous in these areas.

Among the voucher specimens for *L. glabrous* ($n = 16$, Raven & Tai, 1979), a collection from Fort Bend Co., Texas (*Raven 19398*), with short capsules and seed-surface cells in parallel columns transversely elongate to the seed length, has been placed under subsp. *brachycarpa* in Table 2. Another collection (*Raven 19405*) from the same county is preserved in two duplicate specimen sheets: one of them (FLAS) belongs to subsp.

TABLE 3. *Strains of* Ludwigia *used in artificial hybridization experiments.*

L. alata (ALA)[a]
- (a) Franklin Co., Florida, *Godfrey 70575.*
- (b) Levy Co., Florida, *Dille 392.*

L. curtissii (CUR)
- (a) Hillsborough Co., Florida, *Dille 435.*
- (b) Martin Co., Florida, *Peng 4199.*
- (c) Collier Co., Florida, *Peng 4231.*
- (d) Collier Co., Florida, *Peng 4283.*
- (e) Palm Beach Co., Florida, *Popenoe 1962.*
- (f) Franklin Co., Florida, *Godfrey 71148.*

L. glandulosa subsp. *glandulosa* (GLA)
- (a) Santa Rosa Co., Florida, *Dille 412.*
- (b) Emanuel Co., Georgia, *Peng 4013.*
- (c) Columbus Co., North Carolina, *Broome 897.*
- (d) Columbus Co., North Carolina, *Broome 865.*

L. glandulosa subsp. *brachycarpa* (BRA)
- (a) Cameron Parish, Louisiana, *Peng 4367.*

L. lanceolata (LAN)
- (a) Highlands Co., Florida, *Dille 370.*
- (b) Highlands Co., Florida, *Peng 4193.*

L. linearis (LIE)
- (a) Jasper Co., South Carolina, *Dille 350.*
- (b) St. Tammany Parish, Louisiana, *Dille 420.*
- (c) Jasper Co., South Carolina, *Peng 3935.*
- (d) Emanuel Co., Georgia, *Peng 4023.*

L. linifolia (LIF)
- (a) Municipio Huimanguillo, Tabasco, Mexico, *Cowan 2632.*
- (b) Hillsborough Co., Florida, *Dille 427.*
- (c) Wakulla Co., Florida, *Godfrey 77091.*
- (d) Franklin Co., Florida, *Peng 4343.*

L. microcarpa (MIC)
- (a) Clay Co., Florida, *Dille 359.*
- (b) Jackson Co., Florida, *Godfrey 77093.*
- (c) Jones Co., North Carolina, *Peng 3800.*
- (d) Franklin Co., Florida, *Peng 4348.*

L. pilosa (PIL)
- (a) Horry Co., South Carolina, *Dille 342.*
- (b) Hancock Co., Mississippi, *Dille 419.*
- (c) Emanuel Co., Georgia, *Peng 4025.*

L. polycarpa (POL)
- (a) Franklin Co., Missouri, *Dille 328.*
- (b) Franklin Co., Missouri, *Dille 436.*
- (c) Lincoln Co., Missouri, *Dille 443.*

L. simpsonii (SIM)
- (a) Collier Co., Florida, *Dille 378.*
- (b) Sarasota Co., Florida, *Dille 383.*
- (c) Collier Co., Florida, *Peng 4234.*
- (d) Collier Co., Florida, *Peng 4246.*
- (e) Sarasota Co., Florida, *Peng 4313.*

L. sphaerocarpa (SPH)
- (a) Jasper Co., South Carolina, *Dille 348.*
- (b) Wakulla Co., Florida, *Dille 401.*
- (c) Wakulla Co., Florida, *Dille 402.*

TABLE 3. *Continued.*

L suffruticosa (SUF)
- (a) Hillsborough Co., Florida, *Dille 423.*
- (b) Hillsborough Co., Florida, *Dille 424.*
- (c) Hillsborough Co., Florida, *Dille 434.*

[a] Three-letter abbreviations are used for each taxon in Tables 3–14 and Figures 5, 6, 21, 22.

brachycarpa; the other (DS), however, is a mixture of two plants of subsp. *L. glandulosa* with one of subsp. *brachycarpa*. This is arbitrarily placed under subsp. *brachycarpa* in Table 2; both taxa have the same chromosome number.

The following collections counted by Raven & Tai (1979) as $n = 16$ are considered to represent hybrid populations of *L. pilosa* × *L. sphaerocarpa* and have been excluded from Table 2: Florida: Highlands Co., *Raven 18683* (as *L. pilosa*); Clay Co., *Raven 18680* (as *L. sphaerocarpa*); Columbia Co., *Raven 18634* (as *L. sphaerocarpa*). South Carolina: Beaufort Co., *Raven 18716* (as *L. sphaerocarpa*); Colleton Co., *Raven 18718* (as *L. sphaerocarpa*).

Experimental Hybridization

An extensive artificial hybridization program was carried out among members of sect. *Microcarpium* with the following objectives: (1) to determine chromosome homologies and relationships of taxa with the same and with different ploidy levels; (2) to study whether chromosome repatterning has played a role in the diversification of the species; (3) to study the genetic isolating mechanisms that may have permitted preservation of the genetic integrity of the taxa; and (4) to examine the variation pattern generated by hybridization, which is useful in studying natural hybrid populations.

Over 1,000 reciprocal crosses have been made among the 12 species and one additional subspecies of sect. *Microcarpium*. *Ludwigia ravenii* and *L. stricta* were not included because living plants were not available at the time these studies were conducted. Most of the attempted crosses resulted in seed set. Some of the failures probably resulted from damage by the sharp forceps used to emasculate the flowers and to transfer the pollen to these usually small-flowered plants. In these cases, additional attempts were made using either the same or different parental strains, and seed set was usually obtained. The only consistent failure occurred in some crosses involving *L. microcarpa* as pollen parent. This species is the smallest in

TABLE 4. *Percentages of stainable pollen in hybrids between species of* Ludwigia *sect.* Microcarpium. Ludwigia pilosa *group, diploid group, and* L. curtissii *complex are separated by lines.*

	♂												
♂	ALA	BRA	GLA	LAN	PIL	POL	SPH	SUF	LIE	LIF	MIC	SIM	CUR
ALA	·	·	47	62	52	53	71	63	1	0	·	·	·
n = 24													
BRA	·	·	97	56	·	67	·	·	·	·	·	·	·
n = 16													
GLA	46	96	·	·	·	·	·	62	2	·	0	3	·
n = 16													
LAN	64	·	·	·	96	83	95	95	·	·	0	11	4
n = 16													
PIL	43	87	84	75	·	·	98	78	·	0	·	·	6
n = 16													
POL	43	·	·	83	83	·	·	·	·	0	·	·	25
n = 16													
SPH	47	·	·	87	·	·	·	·	1	·	·	15	17
n = 16													
SUF	61	·	·	·	·	·	·	·	·	·	·	·	·
n = 16													
LIE	·	·	·	·	·	0	1	·	·	48	·	·	·
n = 8													
LIF	·	·	0	1	·	·	·	·	47	·	·	·	·
n = 8													
MIC	15	·	2	·	·	13	4	·	0	6	·	9	2
n = 8													
SIM	20	·	·	·	·	·	·	·	·	·	·	·	81
n = 24													
CUR	·	·	5	·	·	32	31	9	0	10	2	89	·
n = 32													

stature and in flower size and has the shortest style and smallest pollen grains among members of sect. *Microcarpium*. The few ovulate parents that failed to set seed in these crosses were of taxa with styles 3–6 times longer than those of *L. microcarpa*. Rather than genetic disharmony, it is likely that these failures were due to the improbability of small grains containing sufficient food reserves to support pollen tube growth through long styles (review in Lee, 1978), since the reciprocal crosses using shorter-styled *L. microcarpa* as ovule parent invariably set seeds.

As all plants in sect. *Microcarpium* are self-compatible and modally autogamous, self-pollination sometimes interfered with the hybridizations, especially in crosses involving a small-flowered ovulate parent. Part of the seed set may thus reflect a small to moderate amount of self-pollination despite regular emasculation. The seedlings resulting from self-pollination, however, can almost always be distinguished from those resulting from hybridization by the leaf color or shape, or even by growth rate, when both types of seedlings were grown together.

The strains utilized in the crossing experiments are presented in Table 3. The results of the artificial hybridizations are summarized in Figure 5 and indicate three more or less interfertile groups in sect. *Microcarpium*: (1) all the tetraploid taxa plus *L. alata*, a hexaploid; (2) *L. linearis* and *L. linifolia*, both diploid species; and (3) the *L. curtissii* complex (hexa- and octoploids). Data on the percentages of stainable pollen in hybrids (Table 4) support this interpretation.

The crossing results will be discussed in the following order: (I) the diploid group (including *L. linearis*, *L. linifolia*, and *L. microcarpa*); (II) the *L. pilosa* group (including all tetraploids and a single hexaploid, *L. alata*); (III) the *L. curtissii* complex (including *L. curtissii* and *L. simpsonii*); (IV) crosses between the diploid group and the *L. pilosa* group; (V) crosses between the diploid group

♀/♂	ALA	BRA	GLA	LAN	PIL	POL	SPH	SUF	LIE	LIF	MIC	SIM	CUR
ALA n=24			◉	◉	◉	◉	◉	◉	○	○	—	X	X
BRA n=16			●	◉		◉			X	X		X	
GLA n=16	◉	●		◉	•	●	•	◉	○	X	○	⊙	
LAN n=16	◉	•	•		●	●	●	●	X	X	○	○	○
PIL n=16	◉	●	●	●		•	●	●	X	○	—		⊙
POL n=16	◉			●	●		•	•	X	○			⊙
SPH n=16	◉		•	●	•	•		•	○	X	X		⊙
SUF n=16	◉		•	•	•	•	•		X	X	—		
LIE n= 8	X			X	X	○	○	X		◉	—		○
LIF n= 8	X	X	○	○	X	X	X	X	◉		—	X	X
MIC n= 8	○		○	X	X	○	○		○	○		⊙	○
SIM n=24	⊙			X	X			X	○	⊙	⊙		●
CUR n=32		X	⊙	X	X	⊙	⊙	○	○	X		●	

● F_1 hybrids producing abundant seeds (usually >80% of potental number)

◉ F_1 hybrids producing fewer seeds (e.g., often about 1/2 of potential number)

⊙ F_1 hybrids producing very few seeds (< 5% of potential number)

○ F_1 hybrids flowering, but setting no seeds

X F_1 hybrid seed formed failed to germinate or died soon after germination

• Hybridization resulted in abundant seed set , but seeds not sown

— Hybridization failed to result in seed set

FIGURE 5. Summary of artificial hybridization between species of *Ludwigia* sect. *Microcarpium*. *Ludwigia pilosa* group, diploid group, and *L. curtissii* complex are separated by lines.

TABLE 5. *Meiotic configurations and pollen stainability in interspecific* Ludwigia *diploid hybrids.*

Hybrid Combination	Modal Meiotic Configuration Observed	Number of Cells with Modal Configuration/ Total Number of Cells Examined	Pollen Stainabilty (%)
LIE d × LIF c	1IV + (4–6)II + (4–0)I	6/20	48
LIF c × LIE d	1IV + (5–6)II + (2–0)I	5/9	47
MIC b × LIE c	(0–1)II + (16–14)I	5/10	0
MIC b × LIF c	(2–3)II + (12–10)I	4/4	6

and the *L. curtissii* complex; and (VI) crosses between the *L. pilosa* group and the *L. curtissii* complex.

HYBRIDS WITHIN THE DIPLOID GROUP (*LUDWIGIA LINEARIS*, *L. LINIFOLIA*, AND *L. MICROCARPA*)

These interspecific crosses resulted in significant seed set except for a few cases in which *L. microcarpa* was pollen donor. As suggested earlier, these failures were probably due to the inability of the small pollen grains of *L. microcarpa* to grow through the long styles of *L. linearis* and *L. linifolia*. Four hybrid combinations were obtained (Table 5) and grown to flowering.

Meiotic analysis and pollen stainability

Meiosis was normal in the examined natural populations of the diploid parental strains. The chromosomes regularly formed eight bivalents, with no univalents or multivalents observed. In the reciprocal crosses between *L. linearis* and *L. linifolia*, meiotic configurations ranged from eight bivalents to four bivalents and eight univalents; a ring or chain quadrivalent was observed in 11 of the 29 cells examined. In a single cell, a hexavalent was observed; trivalents were observed occasionally. In the translocation heterozygote, chiasma frequency and quadrivalent frequency are interdependent to a certain extent, as McCollum (1958) suggested. If chiasmata are formed in each of the four paired arms of the cross-shaped configuration at pachytene, then the four chromosomes form a ring at metaphase I; progressively lower numbers of chiasmata will give a chain of three plus a univalent, two bivalents, or four univalents. The observation of the quadrivalent in meiosis of the diploid hybrid indicates that the strains of *L. linearis* and *L. linifolia* hybridized differ by at least one reciprocal translocation, which may help to account for the inviability in about half of the pollen in the hybrid. This is the first demonstration of a reciprocal translocation in *Ludwigia*. The interpretation of the single cell recorded as having a hexavalent is uncertain, and the observation should be confirmed.

The reciprocal hybrids between *L. linearis* and *L. linifolia* produced many aborted seeds. However, *L. linearis* has the highest number of seeds per capsule (about 700) among species of sect. *Microcarpium*. F_1 plants resulting from reciprocal crosses involving *L. linearis* and *L. linifolia* were also very fecund, and their output of viable seeds per capsule was therefore quite substantial, although reduced. An F_2 family of nearly 100 plants was grown for each of the reciprocal crosses. Some 25% of these plants died in the seedling stage, and another 25% were very weak. About 10–15% of the F_2s flowered. Pollen stainability of the seven F_2 plants examined ranged from 1 to 52% (1, 7, 10, 15, 22, 32, 52%). None of the F_2s that survived were as vigorous and floriferous as the F_1 plants.

In diakinesis and first metaphase of *L. microcarpa* × *L. linearis* and *L. microcarpa* × *L. linifolia* univalents predominated. The bivalents ranged from 0 to 3, the rest being univalents. Heteromorphic bivalents were observed in at least a few cells. The paired chromosomes were sometimes held together by a matrix connection instead of by a chiasma. However, a maximum of three true bivalents, held together by chiasmata, were observed in a few cells. The few paired chromosomes did not always line up in the equatorial plane, more often being randomly placed like univalents. Micronuclei were present at the tetrad stage. Pollen grains were shed both singly and as (loose) tetrads. Their sizes varied. The stainability was 0% in *L. microcarpa* × *L. linearis* and 6% in *L. microcarpa* × *L. linifolia*, which seemed to accord with the fact that no or one bivalent(s) were observed

in the former and two or three were observed in the latter.

Morphology of the hybrids

Reciprocal hybrids between *L. linearis* and *L. linifolia* resembled each other morphologically. They were vigorous and floriferous. In overall habit and capsule shape they were more similar to *L. linearis*. In floral details, however, the hybrids were intermediate between the parents. For example, the divided sporogenous tissue in the anthers, which is characteristic of *L. linearis*, also appeared in the hybrids, although the packets were fewer and shallower. Seed set was variable from capsule to capsule, but was generally fairly high.

Ludwigia microcarpa, a small, highly autogamous herb with minute, apetalous flowers and obovate-spatulate to spatulate leaves, differs from both *L. linearis* and *L. linifolia* in nearly every morphological aspect. Hybrid combinations between *L. microcarpa* and *L. linearis* and those involving *L. microcarpa* and *L. linifolia* were vigorous and morphologically intermediate between the parents. Both produced small flowers with four yellow petals and reached the maximum height of the taller parents. Hybrids between *L. microcarpa* and *L. linearis* were about 70 cm tall and had very narrowly elliptic leaves, whereas those between *L. microcarpa* and *L. linifolia* were about 35 cm tall and had oblanceolate to narrowly obovate leaves. Neither hybrid set fruit—the ovaries simply withered.

HYBRIDS WITHIN THE *LUDWIGIA PILOSA* GROUP

Parental strains of this group consisted of one hexaploid species, *L. alata* ($n = 24$), and seven tetraploid entities ($n = 16$). All interspecific reciprocal crosses attempted invariably yielded abundant seed. As many hybrid combinations as greenhouse space and time allowed were grown (Fig. 5; Tables 6, 7). Hybrid seedlings were nearly always vigorous. Occasionally a few plants assumed stunted growth or exhibited some morphological abnormality. These are considered to represent chance combinations of disharmonious genotypes from the two parents. For each hybrid combination, only the healthy F_1 individuals were grown to maturity for study.

Meiotic analysis and pollen stainability

Meiosis was normal in all parental strains of the tetraploid species as well as in *L. alata*, the only hexaploid in this group. From diakinesis to metaphase I, 16 and 24 bivalents, respectively, were invariably observed in these taxa; no univalents or multivalents were detected. Disjunction and microspore formation also appeared to be normal. The pollen stainability of the parental strains was 95–100%.

As all the hybrids between tetraploid taxa exhibited a similar pattern of meiotic behavior, the data for these are pooled and discussed together. Hybrids between the tetraploid taxa and *Ludwigia alata* (hexaploid) were likewise similar with respect to meiotic behavior; these data will also be treated as a group.

(1) Hybrids between tetraploid taxa
(Table 6)

In very many cases, meiosis in these hybrids appeared normal, exhibiting 16 bivalents from diakinesis to metaphase I. The majority of cells exhibited complete chromosome pairing, but 2–6 (very rarely 8–10) univalents were often encountered in prepared slides. In many cases these univalents appeared to have resulted from precocious separation of the bivalents (probably because no chiasmata developed). This was suggested by their shapes and nonrandom locations on the metaphase plates. Nevertheless, meiotic anaphase I was generally regular, and no micronuclei were observed in the tetrad stage. Occasionally bivalents had a slightly stretched or attenuated appearance. This stretching may result from a failure of terminal chiasmata to disjoin properly (Grant, 1952). A few cells with 2–3 bivalents associated side by side were seen. This association is apparently not due to chiasmata but rather to the "sticky" matrix bands typical of the "pseudobivalents" in hybrids of other taxa (e.g., *Bromus*, Walters, 1954).

Despite the few meiotic irregularities observed, the pollen stainability of the tetraploid hybrids was generally high (Tables 4, 6). Of the 17 hybrid combinations examined, 6 had estimated pollen stainabilities of 95–98%. With a few exceptions, most other hybrids exhibited 75–87% stainable pollen.

Two hybrids failed to flower in the closed greenhouse: *L. glandulosa* subsp. *brachycarpa* × *L. lanceolata* and *L. sphaerocarpa* × *L. glandulosa* subsp. *glandulosa*. These were then moved to an open, netted greenhouse. Here, the hybrids between *L. sphaerocarpa* × *L. glandulosa* subsp. *glandulosa* produced a single flower; its pollen stainability was not studied. The hybrid *L. glandulosa* subsp. *brachycarpa* × *L. lanceolata* was very vigorous and produced many flowers two

TABLE 6. *Meiotic configurations and pollen stainability in interspecific* Ludwigia *tetraploid hybrids.*

Hybrid Combination	Modal Meiotic Configuration Observed	Number of Cells with Modal Configuration/ Total Number of Cells Examined	Pollen Stainability (%)
BRA × GLA b	16II	10/10	97
BRA × LAN a	15II + 2I	4/12	56
BRA × POL c			67
GLA b × BRA	16II	15/25	96
GLA d × POL b	Fruiting specimen showed plump capsules with abundant seeds.		
GLA b × SUF b			62
LAN a × PIL a	16II	12/13	96
LAN a × POL c	16II	14/19	83
LAN a × SPH b	16II	9/13	95
LAN a × SUF a	14II + 4I	4/6	95
PIL c × BRA	16II	5/5	87
PIL b × GLA d	(14–16)II + (4–0)I	5/6	84
PIL b × LAN a	16II	14/15	75
PIL b × SPH b	15II + 2I	5/7	98
PIL b × SUF a			78
POL c × LAN a			83
POL c × PIL b			83
POL c × SPH b	Fruiting specimen showed plump capsules with abundant seeds.		
SPH b × GLA c	Fruiting specimen showed plump capsules with abundant seeds.		
SPH c × LAN a			87

months after transfer. Its pollen stainability was estimated to be only 56%, significantly lower than one would expect, since this plant has as much chromosome pairing (13–16 pairs) as other hybrids that exhibited 75–98% stainable pollen.

Jones (1976) noted that environmental factors may exert considerable influence on production of presumably normal stainable pollen. She found that plants of some *Aster* species moved out from the greenhouse to the field consistently yielded significantly reduced fractions of normal pollen. Whether this is the case for *L. glandulosa* subsp. *brachycarpa* × *L. lanceolata* remains to be confirmed by growing them in a closed experimental greenhouse. It is also possible that the low observed pollen stainability is the result either of genic interaction between the parents or of the pronounced meiotic irregularities discussed earlier. However, the present sample size is not large enough to establish that hybrids from the cross *L. glandulosa* subsp. *brachycarpa* × *L. lanceolata* indeed exhibit a higher degree of meiotic irregularity than other hybrids.

Reciprocal hybrids between tetraploid taxa generally exhibited similar values for pollen stainability (Tables 4, 6). Different values were obtained for the reciprocal crosses between *L. lanceolata* and *L. pilosa* (96% and 75%), but different strains of *L. pilosa* were used in these crosses.

These data suggest that the tetraploid species in sect. *Microcarpium* in general have high chromosome homology, and complete pairing is frequently observed in almost all interspecific hybrids. The few meiotic irregularities occasionally observed (a few univalents, slightly attenuated bivalents, and sticky bivalents) have little effect on the hybrid fertility (as estimated by pollen stainability). This was further substantiated by abundant seed sets of all the hybrids.

A small family of vigorous F_2 individuals was reared for two randomly selected hybrids: *L. pilosa* × *L. glandulosa* subsp. *glandulosa* (17 plants) and *L. lanceolata* × *L. suffruticosa* (19 plants). These populations were small, owing to the limited space and time available to handle them. They generally received less care than the F_1s. Therefore, the several plants that were weak or died may not necessarily reflect F_2 weakness or breakdown. For example, the organic potting soil that was used was easily spoiled since all pots were continuously kept in standing water and were sprayed with insecticide periodically. Had the potting soil not been changed as needed, the plants might have remained vegetative, become weak, or even died.

TABLE 7. *Meiotic configurations and pollen stainability in hybrids resulting from crosses between tetraploid* Ludwigia *species and the hexaploid species (*L. alata*).*

Hybrid Combination	Modal Meiotic Configuration Observed	Number of Cells with Modal Configuration/ Total Number of Cells Examined	Pollen Stainability (%)
ALA a × GLA c	16II + 8I	10/16	47
GLA d × ALA a	(15–16)II + (10–8)I	7/9	46
ALA a × LAN a	16II + 8I	15/29	62
LAN a × ALA a	(15–16)II + (10–8)I	4/4	64
ALA a × PIL a	(12–16)II + (16–8)I	10/10	52
PIL a × ALA a	(15–16)II + (10–8)I	6/9	43
ALA a × POL a			50
ALA a × POL b			56
POL a × ALA a	(11–15)II + (18–10)I	8/11	43
ALA a × SPH b	16II + 8I	13/22	71
SPH a × ALA a	(13–16)II + (14–8)I	2/7	47
ALA a × SUF a	16II + 8I	5/6	63
SUF c × ALA a	(14–16)II + (12–8)I	7/7	61

All F_2s that did grow were vigorous. In the cross *L. pilosa* × *L. glandulosa* subsp. *glandulosa*, the only individual that flowered was quite unlike either the parents or the F_1s in morphology but had 91% stainable pollen. In *L. lanceolata* × *L. suffruticosa*, about 10 F_2 individuals flowered, some of which resembled the intermediate F_1s, whereas others were similar to *L. lanceolata*. Pollen stainability was quite different from plant to plant, ranging from 1% to 97% (1, 12, 51, 63, 74, 97%) in the six plants studied. Meiosis of an F_2 individual (pollen stainability not assessed) was nearly normal. Complete chromosome pairing was nearly always exhibited, although 2–4 univalents were occasionally seen. Some of the univalents were formed as a result of precocious disjunction of the bivalents.

(2) Hybrids between tetraploid taxa and hexaploid species (Table 7)

The hexaploid *L. alata* ($n = 24$) was crossed reciprocally to nearly all of the tetraploid ($n = 16$) taxa. All crosses resulted in vigorous, floriferous, pentaploid hybrids. Meiosis in the F_1 individuals typically exhibited a maximum of 16 bivalents and 8 scattered univalents, although exceptional configurations of 17 bivalents and 6 univalents were observed in *L. alata* × *L. lanceolata* (one cell), *L. alata* × *L. sphaerocarpa* (four cells), and *L. pilosa* × *L. alata* (one cell). One or two trivalents were seen occasionally in diakinesis and metaphase I of the following hybrids: *L. alata* × *L. glandulosa* subsp. *glandulosa* (one trivalent in two cells; two trivalents in one cell), *L. alata* × *L. lanceolata* (one trivalent in four cells; two trivalents in one cell), *L. alata* × *L. sphaerocarpa* (two trivalents in one cell), and *L. polycarpa* × *L. alata* (one trivalent in two cells; two trivalents in one cell). Groups of 2–4 bivalents (often of similar shape and size) "sticking" to one another sidewise were observed in meiotic metaphase but not in diakinesis. These associations were obviously not a result of chiasmata formation. Eight univalents would normally be expected in these hybrids. Observations of additional univalents at metaphase I either represent precociously separated bivalents or chromosomes which have no homologue. Present observations suggest that in most cells which had more than eight univalents, these "extras" are often precociously separate bivalents, judged by their shapes and locations.

Pseudobivalents (Walters, 1954) composed of two univalents held together by matrix bands were seen at least in a few metaphase I cells. One to six (rarely to ten) bivalents of stretched appearance were also seen occasionally. Some metaphase I cells in *L. alata* × *L. suffruticosa* were peculiar in having attenuated ends on the bivalents. Lagging univalents were uniformly found in meiotic anaphase I and II of all hybrids, and micronuclei occurred in nearly all the sporads.

Pollen stainability was in the range 43–71%, rather high for pentaploid hybrids exhibiting the above meiotic irregularities. All F_1 plants produced

some aborted and some viable seeds in the plump capsules. An F_2 family (*L. glandulosa* subsp. *glandulosa* × *L. alata*) of 32 vigorous plants was grown and many individuals probably flowered but were not studied in detail. One morphologically intermediate plant was examined, however, and had 31% stainable pollen.

Morphology of the hybrids

Because of an apparently chance distribution of dominance between parents, some hybrids are likely to possess some characters of one parent, some of the other, and some (due to incomplete dominance or polygenic inheritance) that are intermediate (Stace, 1975). The net result is that hybrids are nearly always intermediate in overall morphology between the two parents. This is the situation within sect. *Microcarpium*. Genomic interaction resulting in a new character state has not been observed in these plants. With the exceptions of *L. glandulosa* subsp. *glandulosa* × *L. polycarpa* and *L. sphaerocarpa* × *L. glandulosa* subsp. *glandulosa*, all hybrids were very vigorous and flowered over a period of at least a month. Even in these two exceptional hybrids, if additional seeds were sown, or if different parental strains were crossed, vigorously growing hybrids would, I believe, be expected. This is because of the occurrence of a natural hybrid population of *L. sphaerocarpa* × *L. glandulosa* subsp. *glandulosa*. As there are very many naturally occurring hybrid populations between members of the *L. pilosa* group, it is valuable to discuss the morphology of artificial hybrids between these taxa. Since all reciprocal hybrids resembled each other, the discussion of characters that follows is limited to crosses in only one direction.

(1) Tetraploid hybrids

Ludwigia glandulosa subsp. *glandulosa* × *L. polycarpa*. These were dwarfs 5–20 cm in height at maturity, with an ascending habit. Despite their abnormal appearance, all produced a few flowers, set plump capsules with abundant seeds, and survived in the experimental greenhouse for two consecutive years. In general they were intermediate in vegetative and reproductive features, especially in shapes and sizes of floral parts and capsules. Like their parents, the hybrids shed pollen grains as tetrads and had seed-surface cells in parallel columns elongate to the seed length.

Ludwigia glandulosa subsp. *glandulosa* × *L. glandulosa* subsp. *brachycarpa*. The intermediacy of these hybrids was apparent in capsule size (4.5–5.5 mm long) and seed surface, which exhibited a mixture of columnar cells elongate and transversely elongate to the seed length (Fig. 6).

Ludwigia lanceolata × *L. pilosa*. The parents did not differ in seed-surface cell pattern or in the way their pollen grains were shed. The hybrids showed intermediate morphology in all aspects. Diagnostic characters include hirtellous pubescence and oblong-obpyramidal, 4-angled, unwinged capsules.

Ludwigia lanceolata × *L. polycarpa*. The hybrids were morphologically intermediate between the parents. Diagnostic characters include the 4-angled capsules and seed-surface cells (Fig. 7) basically similar in orientation to those of *L. polycarpa* (Table 1) but arranged in shorter columns. Some of them appeared more or less isodiametric in shape.

Ludwigia lanceolata × *L. sphaerocarpa*. The hybrids were again intermediate. The obpyramidal capsules were puberulent and very slightly winged, and the seed surface was composed of some more or less isodiametric cells and numerous, variously oriented short columnar cells (Fig. 8).

Ludwigia lanceolata × *L. suffruticosa*. The hybrids were generally intermediate in morphology. The stems were much branched as in *L. lanceolata*, but with a slightly congested and branched terminal inflorescence. The lower margins of sepals, bracteoles, and peduncles were slightly hirtellous. The capsules were weakly 4-winged and the seed-surface cells were subisodiametric as in both parents. Pollen grains were shed singly as in *L. suffruticosa*.

Ludwigia pilosa × *L. glandulosa* subsp. *glandulosa*. These hybrids were similar to *L. glandulosa* subsp. *glandulosa* in having subcylindric capsules, which were, however, somewhat shorter (5–5.5 mm long). Otherwise the plants were intermediate in general appearance, pubescence, and shape and size of floral parts. Seed-surface cells (Fig. 9) were predominantly columnar and elongate to the seed length like those of *L. glandulosa* subsp. *glandulosa* (Fig. 2). Yet few columnar cells were transversely elongate or oblique to the seed length. Some subisodiametric cells characteristic of *L. pilosa* were also present.

Ludwigia pilosa × *L. sphaerocarpa*. The hybrids were intermediate in pubescence (being densely strigillose) and shape and size of floral parts. Seed surface pattern (Figs. 10, 11, 12) was irregular and variable within the same capsule. A small yellow petal was observed in a single flower. Vestigial

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petals, however, occur occasionally in both parental species.

Ludwigia pilosa × *L. suffruticosa*. The hybrids were intermediate in leaf shape and habit, were similar to *L. pilosa* in being hirtellous, and resembled *L. suffruticosa* in having a congested inflorescence. Fruiting specimens were not studied.

Ludwigia pilosa × *L. glandulosa* subsp. *brachycarpa*. The hybrids were similar to *L. pilosa* × *L. glandulosa* subsp. *glandulosa* in general, although they were less robust and had shorter subcylindric capsules 3–3.5 mm in length. The plants were strigillose. Their seed surface was composed predominantly of columnar cells, which were transversely elongate to the seed length (Fig. 13) as in *L. glandulosa* subsp. *brachycarpa*. Some more or less isodiametric cells typical of *L. pilosa* were also present, however. Also, columnar cells that were either parallel to the seed length or randomly oriented were observed.

Ludwigia polycarpa × *L. pilosa*. Hybrids were morphologically intermediate. Diagnostic features included the overall strigillose pubescence and the cubic-turbinate capsule with long bracteoles. These plants exhibited seed-surface cell patterns similar to those of *L. pilosa* × *L. glandulosa* subsp. *glandulosa* (Fig. 9).

Ludwigia sphaerocarpa × *L. glandulosa* subsp. *glandulosa*. The hybrids were not as vigorous as other hybrid combinations involving members of this group. The plants were reddish and less than 20 cm high. They were glabrous except for the fruits, which were sparsely and minutely strigillose. Only a single individual flowered, which later set plump capsules oblong in outline. Seed-surface cell pattern was basically similar to that of *L. glandulosa* subsp. *glandulosa*, but with some irregularities.

Ludwigia glandulosa subsp. *brachycarpa* × *L. lanceolata*. These hybrids were morphologically intermediate between the parents. The plants were nearly glabrous. The capsules were about 3 mm long, elongate obpyramidal, minutely strigillose on the sepal margins, and weakly winged on the angles. The bracteoles were short (about 1.2–1.5 mm long) and, as in *L. glandulosa* subsp. *brachycarpa*, the seed surface (Fig. 14) consisted mostly of columnar cells elongate transversely to the seed length. Some cells were arranged parallel to the seed length. Isodiametric cells, as characteristic of *L. lanceolata*, were uncommon.

(2) Pentaploid hybrids

Ludwigia alata × *L. glandulosa* subsp. *glandulosa*. These hybrids were similar to *L. alata* in habit, leaf shape, and in having winged capsules. Intermediate characters included capsule shape (elongate obpyramidal) and pollen grains shed singly and as tetrads. The seed surface in the hybrid consisted mainly of columnar cells elongate transversely to the seed length, as in *L. alata*, although the cells were much smaller and were sometimes more nearly isodiametric than on seeds of that species (Fig. 15). Columnar cells elongate parallel to the seed length and similar to those of *L. glandulosa* subsp. *glandulosa* were also observed.

Ludwigia alata × *L. lanceolata*. The parental species were themselves very similar in floral morphology and habit. The hybrids can be identified only by seed surface (Fig. 16), which consists of a mixture of columnar cells elongate transversely to the seed length, as in *L. alata*, and more or less isodiametric cells characteristic of *L. lanceolata*. Pollen grains were shed singly as in *L. alata*.

Ludwigia alata × *L. pilosa*. These hybrids were generally intermediate. They were neither glabrous as in *L. alata* nor hirsute as in *L. pilosa*, but were minutely villous all over. The capsules, like those of *L. alata*, were winged. Seed-surface cell pattern (Fig. 17) was similar to that of *L. alata* × *L. lanceolata*. Pollen grains were shed mostly as tetrads, although single grains were seen occasionally.

Ludwigia alata × *L. polycarpa*. With their winged capsules, the hybrids were more similar to *L. alata* in general appearance. They had the minutely strigillose leaf margins characteristic of *L. polycarpa*, however. Conspicuously intermediate characters include: sepal shape, seed surface

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FIGURES 6–19. Photographs of seeds obtained from experimental hybrids in *Ludwigia* sect. *Microcarpium*. —6. *L. glandulosa* subsp. *brachycarpa* × *L. glandulosa* subsp. *glandulosa*. —7. *L. lanceolata* × *L. polycarpa*. —8. *L. lanceolata* × *L. sphaerocarpa*. —9. *L. pilosa* × *L. glandulosa* subsp. *glandulosa*. —10–12. *L. pilosa* × *L. sphaerocarpa*. —13. *L. pilosa* × *L. glandulosa* subsp. *brachycarpa*. —14. *L. glandulosa* subsp. *brachycarpa* × *L. lanceolata*. —15. *L. alata* × *L. glandulosa* subsp. *glandulosa*. —16. *L. lanceolata* × *L. alata*. —17. *L. alata* × *L. pilosa*. —18, 19. *L. alata* × *L. polycarpa*. Scale bar = 0.5 mm.

pattern in which columnar cells were randomly oriented (Figs. 18, 19), and pollen grains shed as loose tetrads.

Ludwigia alata × *L. sphaerocarpa*. These hybrids were similar to *L. alata* in habit and in having winged capsules. However, they shed pollen grains as loose tetrads, and their capsules were strigillose, both intermediate character states. Seed-surface cell pattern is not of diagnostic value here, as both parents were similar in this respect.

Ludwigia alata × *L. suffruticosa*. These hybrids generally resembled *L. alata*. The flowers were loosely arranged along the apices of the stems, and the capsules were winged and glabrous. The seed-surface cell pattern was intermediate between that of the two parents, being similar to that shown in Figure 16. The hybrids shed pollen grains singly, as did both parents.

In summary, artificial hybrids between members of the *L. pilosa* group were easily produced and were nearly always vigorous and floriferous. Morphologically, F_1 plants were more or less intermediate between the parents. The only character that exhibited some consistent degree of dominance is the winged capsules of *L. alata*. When tetraploid species with rounded capsules were crossed with *L. alata*, the resultant hybrids invariably had distinctly winged capsules. In contrast, when *L. lanceolata*, a tetraploid with winged capsules, was crossed with tetraploid species having rounded capsules, the F_1 individuals usually exhibited intermediate capsule shape—their capsules were 4-angled or at most slightly winged. Therefore, it seems that in hybrids involving *L. alata*, the apparent dominance of the winged-capsule character may be due to a multiple dose of genetic information received from this hexaploid.

The single most important diagnostic character for hybrids within the *Ludwigia pilosa* group is the shape and size of the capsules. Other diagnostic characters include overall pubescence, seed-surface pattern, and to some extent whether pollen grains are shed singly or as tetrads. This pollen character is of limited value, as most species in the *L. pilosa* group shed pollen as tetrads. When one of the taxa that do shed pollen singly was used as a parent, loose tetrads or a mixture of single and tetrad pollen grains were commonly found in the hybrids. This pattern was also clearly shown by the diploid hybrids *L. microcarpa* × *L. linearis* and *L. microcarpa* × *L. linifolia*. Similarly, hybrids between species that differ in their seed-surface pattern always showed a mixture of cell types or cells with intermediate shapes and/or of random orientation. Likewise, a hybrid resulting from crossing a hirtellous species with a glabrous species always showed minutely villous or strigillose pubescence. Examination of the above-mentioned diagnostic characters permits very accurate determination of the parentage involved in hybrids within the *L. pilosa* group.

HYBRIDS WITHIN THE *LUDWIGIA CURTISSII* COMPLEX

Two species, *L. curtissii* ($n = 32$) and *L. simpsonii* ($n = 24$), are included in this complex. Reciprocal hybridizations (Table 8) between them resulted in vigorous, floriferous F_1 individuals.

Meiotic analysis and pollen stainability

Meiosis was normal in both parental species. Diakinesis and metaphase I cells consistently revealed only bivalents. Multivalents were never observed despite the polyploidy of both parents. The two parents have pollen stainability of 97–100%.

Although reciprocal hybrids were made, meiosis was examined only in F_1s in which *L. curtissii* was the ovulate parent and *L. simpsonii* the pollen parent. Of the seven clear, analyzable cells, three were at diakinensis and four in metaphase I. They consistently showed a configuration of 24 bivalents and 8 univalents. Nearly all of the bivalents in the metaphase I cells were regularly rod-shaped, and were oriented on the equatorial plate. The eight univalents were scattered at random along a continuous bipolar spindle; some were probably lost at anaphase I and II, since micronuclei were observed in many sporads. In spite of the presence of scattered univalents, the hybrids produced 89% stainable pollen grains and a moderate number of viable seeds. Eleven F_2 plants grew up to flower. All of them branched profusely and set abundant fruits and seeds. The reciprocal F_1 hybrid, *L. simpsonii* × *L. curtissii*, had a similar value for stainable pollen (81%).

Morphology of the hybrids

Ludwigia curtissii and *L. simpsonii* are often difficult to distinguish; the only consistent diagnostic character available to separate them is the size of mature capsules. Those of *L. simpsonii* are 1.5–2(–2.5) mm long, those of *L. curtissii* (2–) 2.5–4.5 mm long. Artificial hybrids between them had robust, erect stems up to 85 cm high and capsules about 2.5 mm long. Such plants are likely to be identified as *L. curtissii* in the field or the herbarium, although their capsule size is at the

TABLE 8. *Meiotic configurations and pollen stainability of hybrids between members of the* Ludwiga curtissii *complex.*

Hybrid Combination	Modal Meiotic Configuration Observed	Number of Cells with Modal Configuration/ Total Number of Cells Examined	Pollen Stainability (%)
CUR d × SIM d	24II + 8I	7/7	89
SIM e × CUR d			81

lower limit for *L. curtissii*. The hybrid plants set plump capsules with moderate amounts of viable seed.

HYBRIDS BETWEEN THE DIPLOID GROUP AND THE *LUDWIGIA PILOSA* GROUP

Of the 42 hybrid combinations in which reciprocal crossing attempts have been made involving diploid taxa and members of the *L. pilosa* group (Fig. 5, Table 9), only three failed to result in seed set. These crosses involved the small-flowered *L. microcarpa* as pollen parent and one of the larger-flowered species as ovulate parent. Eight of the 39 successful crosses yielded offspring that did not germinate. Of these, 15 resulted in weak F_1 individuals that died soon after germination or remained sterile for the entire season. Only the remaining 15 hybrid combinations flowered successfully, but three of them were weak and died soon after anthesis. The 12 remaining combinations were vigorous and floriferous. Despite this, none of them set any seed. Their ovaries simply turned yellowish and dropped off or were somewhat persistent but shriveled after mechanical self-pollination.

The results of artificially hybridizing the different species of the above two groups ranged from seeds that were unable to germinate to vigorous and floriferous F_1 plants. A few general comments are appropriate here. First, the inability of hybrid seeds to germinate as observed in the present study is not necessarily a reliable indication of reproductive isolation. In some cases, seeds obtained from a particular cross did not germinate the first year, but additional seeds planted the following year did. Seed dormancy, however, is not characteristic of *Ludwigia*, at least of sect. *Microcarpium*. In a few rare cases, hybrid seeds were observed to germinate three or four months after they were sown. These would have been scored as germination failures if the experiments had been terminated after two months, as was generally done.

Second, on several occasions the majority of seeds failed to germinate or produced weak or stunted seedlings, although one or few vigorous and floriferous hybrid individuals grew to maturity. For example, only a single, healthy plant of *L. microcarpa* × *L. alata* was obtained from 18 seeds sown; none of the others germinated. Such failures to germinate are not mentioned in the above discussion, and records were not kept, because the seeds of *Ludwigia* species are very small, and usually 50–200 seeds were sown for each hybrid combination.

Third, weak or sterile hybrids could sometimes be brought to flower if they were grown very carefully or if alternative parental strains were utilized to vary the genetic composition of the hybrids (for examples see Table 9).

Meiotic analysis and pollen stainability

Cytological data are available for ten hybrid combinations. These include seven hybrids resulting from crosses between the tetraploid species and each of the three diploid species, including one reciprocal cross (Table 10), and three hybrids resulting from crosses between *L. alata* (hexaploid) and each of the three diploid species.

(1) Hybrids resulting from crosses between the diploid and tetraploid species

Crosses between diploids and tetraploids would normally be expected to form triploid hybrids. It is most interesting, therefore, that upon sowing 15 seeds resulting from crossing *L. linifolia* ($n = 8$) with *L. lanceolata* ($n = 16$), only 5 plants were obtained, all of which were tetraploid ($2n = 32$). That the diploid *L. linifolia* was used as the ovulate parent suggests that these $2n = 32$ plants were not simply the result of self-pollination in the tetraploid. This chromosome number was apparently produced by the union of an unreduced egg from the diploid *L. linifolia* with a normal sperm nucleus

TABLE 9. *Summary of crossing results between taxa of the diploid group and the* Ludwigia pilosa *group.*

L. linearis as female parent	
LIE b × ALA a	Seeds failed to germinate.
LIE a × LAN a	Plants weak, with reddish leaves, died soon after germination.
LIE a × PIL b	Seeds failed to germinate.
LIE b × POL c	Plants weak, barely flowered; no viable pollen.
LIE a × SPH c	F_1 hybrids vigorous and flowered (see Table 10).
LIE a × SUF b	Low germination percentage; weak plants died when ca. 4 cm high.
L. linearis as male parent	
ALA a × LIE a	F_1 hybrids vigorous and flowered (see Table 11).
BRA × LIE b	Formed a mat in 3-inch pots; remained sterile.
GLA a × LIE a	Plants sterile, about 10–30 cm high.
GLA a × LIE b	Plants sterile, about 5–30 cm high.
GLA a × LIE d	F_1 hybrids vigorous and flowered (see Table 10).
LAN a × LIE a	Died at cotyledon stage.
PIL b × LIE b	Formed 2–4 leaves and died.
POL c × LIE b	Seeds failed to germinate.
SPH c × LIE a	F_1 hybrids vigorous and flowered (see Table 10).
SUF c × LIE b	Some died in cotyledon stage; some produced a few pinkish leaves and soon withered.
L. linifolia as female parent	
LIF b × ALA a	Seeds failed to germinate.
LIF c × ALA b	Seeds failed to germinate.
LIF c × BRA	Cotyledons expanded a month after radicles had protruded; plants weak and died when ca. 1 cm high with 4–6 leaves.
LIF c × GLA c	Seeds failed to germinate.
LIF c × GLA b	Plants weak, mostly died 2–3 cm high; one plant barely flowered and showed 0% stainable pollen.
LIF b × LAN a	F_1 hybrids vigorous and flowered, but with an unexpected chromosome number of $2n = 32$ (see Table 10).
LIF c × PIL b	Very low germination percentage; 3 weak plants obtained, which died when ca. 1 cm high with about 10 leaves.
LIF b × POL c	Seeds failed to germinate.
LIF c × SPH c	Plants weak, sterile, about 5–7 cm high.
LIF b × SUF c	Five out of 20 seeds germinated; all very weak, died soon.
LIF c × SUF a	Numerous seeds germinated, but all died in 4–6-leaved stage.
L. linifolia as male parent	
ALA a × LIF c	F_1 hybrids vigorous and flowered (see Table 11).
BRA × LIF c	Plants remained small, died when ca. 2 cm high.
GLA b × LIF a	Plants weak, with reddish brown leaves, sterile.
LAN a × LIF b	Six out of 30 seeds germinated; all died in seedling stage.
PIL b × LIF b	F_1 hybrids vigorous and flowered (see Table 10).
PIL b × LIF c	Cotyledons reddish, soon withered.
POL c × LIF b	Died in cotyledon stage.
POL c × LIF c	Two weak plants barely flowered and soon died; 0% stainable pollen.
SPH c × LIF c	Plants with pinkish yellow leaves, 10–15 cm high, sterile.
SUF b × LIF b	Seeds failed to germinate.
L. microcarpa as female parent	
MIC a × ALA a	F_1 hybrids vigorous and flowered (see Table 11).
MIC a × GLA b	Plants healthy, flowered when 10–15 cm high.
MIC b × LAN a	Seeds failed to germinate.
MIC a × PIL b	Seeds failed to germinate.
MIC a × POL c	F_1 hybrids vigorous and flowered (see Table 10).
MIC c × SPH c	Plants flowered, but meiosis was not studied; 4% stainable pollen.
L. microcarpa as male parent	
ALA b × MIC a	Crosses failed to set seed.
GLA b × MIC a	F_1 plants healthy and flowered (see Table 10).
LAN b × MIC d	Plants recently grown; healthy, but have not yet flowered.
PIL c × MIC b	Crosses failed to set seed.
SPH a × MIC a	Very low germination rate; soon died.
SUF b × MIC a	Crosses failed to set seed.

TABLE 10. *Meiotic configurations and pollen stainability in seven hybrids resulting from crosses between the diploid with tetraploid species of* Ludwigia *sect.* Microcarpium.

Hybrid Combination	Modal Meiotic Configuration Observed	Number of Cells with Modal Configuration/ Total Number of Cells Examined	Pollen Stainability (%)
L. linearis as one parent			
GLA a × LIE b	(4–7)II + (16–10)I	5/5	2
LIE a × SPH c	(4–7)II + (16–10)I	6/9	1
SPH c × LIE a	(6–7)II + (12–10)I	19/24	1
L. linifolia as one parent			
LIF b × LAN a	8II + 16I[a]	4/8	1
PIL b × LIF b	(6–7)II + (12–10)I	6/6	0
L. microcarpa as one parent			
GLA b × MIC a	(2–6)II + (20–12)I	9/11	0
MIC a × POL c	8II + 8I; 4II + 16I	2/2	13

[a] Hybrids with $2n = 32$, a number apparently produced by the union of an unreduced egg from the diploid (*L. linifolia*) with a normal sperm nucleus from the tetrapoloid (*L. lanceolata*).

from the tetraploid *L. lanceolata*. These plants are, following the terminology of Harlan & deWet (1975), Class I Polyploids. Diakinesis observed in one plant resulting from this cross clearly showed a configuration of 8II + 16I. Configurations at metaphase I, however, ranged from 6II + 20I to 8II + 16I, with two cells each exhibiting a single trivalent. The bivalents were apparently formed between chromosomes of the duplicated *L. linifolia* genome. This unexpected but significant finding suggests strongly that (a) chromosomes in the two genomes in the tetraploid *L. lanceolata* are not homologous and thus remained unpaired; and (b) the genome of *L. linifolia* does not pair with either of the genomes of the tetraploid species when true chromosome homologues are present.

The cytological behavior of all other hybrids generally followed a consistent pattern (Table 10). Although the number of bivalents formed was variable, it never exceeded eight, the haploid chromosome number of the diploid species. The number of bivalents ranged from three to seven in most hybrids, although values ranging from one to six were observed in *L. glandulosa* subsp. *glandulosa* × *L. microcarpa*. The configuration 8II + 8I was observed in only a single cell in *L. microcarpa* × *L. polycarpa*. However, in this metaphase I cell, two of the bivalents are in a somewhat perpendicular orientation to the other six bivalents and appear to be attached univalents. A single trivalent was seen in three of the 33 cells examined of the reciprocal hybrids between *L. linearis* and *L. sphaerocarpa*. One to three heteromorphic bivalents, in which the two chromosomes differed in shape or size, were observed on occasion. Attenuated bivalents and precociously separated bivalents were also seen in some cells. Pseudoassociation resulting from stickiness between two univalents, two bivalents, or one of each, was also observed in a few cases.

It is observed that the two to seven chromosome pairs observed in these diploid × tetraploid hybrids indicate that the diploid genome is homologous with only one of the tetraploid genomes.

The cytological abnormalities and the high proportion of univalents observed in these hybrids are reflected in the low pollen stainability. Six of the seven hybrids had less than 2% stainable pollen; only *L. microcarpa* × *L. polycarpa* had greater than 10% stainable pollen (Table 10), presumably due to chance events. In *L. microcarpa* × *L. polycarpa*, the pollen was shed as single grains and as tetrads, a combination of the characters seen in the two parental taxa. Most stainable pollen, however, was shed as single grains, an observation which cannot be explained at present.

(2) Hybrids resulting from crosses between the diploid and hexaploid species

All hybrids involving diploid and hexaploid taxa were made (Table 11). When *L. alata* was crossed to *L. linearis* and *L. linifolia*, the resulting hybrids typically exhibited a configuration of (1–2)III +

TABLE 11. *Meiotic configurations and pollen stainability in three hybrids resulting from crosses between the diploid and hexaploid species of* Ludwigia *sect.* Microcarpium.

Hybrid Combination	Modal Meiotic Configuration Observed	Number of Cells with Modal Configuration/ Total Number of Cells Examined	Pollen Stainability (%)
ALA b × LIE a	(1–2)III + (4–6)II + (21–16)I	7/12	1
ALA b × LIF c	1III + 6II + 17I	1/6	0
MIC a × ALA b	1IV + (7–10)II + (14–8)I	5/14	15

(4–6)II + (21–16)I in both diakinesis and metaphase I, although a configuration of 8II + 16I was occasionally seen. There is no unequivocal evidence to indicate whether the trivalents represent intergenomic homology in the hexaploid *L. alata* or associations between chromosomes of *L. alata* and those of *L. linearis* or *L. linifolia*. In metaphase I of many cells, one to three bivalents appeared to be attached univalents and were often observed away from the equatorial plate. Other cytological aberrations, including heteromorphic bivalents and elongate, attenuated bivalents, were very common. Normal rod or ring bivalents were infrequent.

In *L. microcarpa* × *L. alata* the modal meiotic configuration was 1IV + (7–10)II + (14–8)I, with maximum pairing of either 1IV + 10II + 8I or 11II + 10I. In the latter case, the quadrivalent was not seen, as it probably separated into two bivalents because of a lack of sufficient chiasmata to hold them together. Rings or chains of four chromosomes were more commonly seen in diakinesis than in metphase I. Trivalents were not noted. It is uncertain whether the quadrivalent indicated that, in addition to the difference in ploidy level, the two species differ by a reciprocal translocation, as the multivalent can also result from associations of one chromosome from *L. microcarpa* and three chromosomes each from one of the three genomes of *L. alata*.

The bivalents observed in the meiotic first metaphase of *L. microcarpa* × *L. alata* were typically normal, being ring- or rod-shaped. Attenuated bivalents were very seldom found. Heteromorphic bivalents or attached univalents were not observed. The reduced occurrence of cytological aberrations and the higher number of chromosome pairings in these hybrids probably account for their higher

TABLE 12. *Summary of crossing results between the diploid species of* Ludwigia *sect.* Microcarpium *and the* L. curtissii *complex.*

L. curtissii as female parent	
CUR a × LIE a	Seeds failed to germinate.
CUR a × LIE b	Most seeds failed to germinate; those that did germinate died at the cotyledon stage.
CUR a × LIE c	Seeds failed to germinate.
CUR e × LIE b	Hybrids stunted at first, vigorous and floriferous eventually.
CUR a × LIF b	Hybrids vigorous and floriferous.
CUR f × MIC a	Hybrids vigorous and floriferous.
L. curtissii as male parent	
LIE b × CUR f	Numerous plants remained in the cotyledon stage and were reddish, four of which flowered ultimately.
LIF b × CUR a	Seeds failed to germinate.
L. simpsonii as female parent	
SIM d × LIE b	Hybrids stunted but flowered.
SIM d × LIF c	Seeds failed to germinate.
SIM e × LIF c	Seeds failed to germinate.
SIM f × LIF c	Seeds failed to germinate.
L. simpsonii as male parent	
LIF c × SIM d	Plants remained small and rosettelike, ca. 10-leaved, 1 cm high 7 months after germination.
MIC d × SIM d	Hybrids vigorous and floriferous.

TABLE 13. *Meiotic configurations and pollen stainability in hybrids resulting from crosses between diploid species of* Ludwigia *sect.* Microcarpium *and the* L. curtissii *complex.*

Hybrid Combination	Modal Meiotic Configuration Observed	Number of Cells with Modal Configuration/ Total Number of Cells Examined	Pollen Stainability (%)
CUR e × LIE b			0
CUR a × LIF b	8II + 24I	13/31	10
CUR a × MIC a	(14–15)II + (12–10)I	5/5	2
LIE b × CUR e			2
MIC b × CUR a			2
MIC d × SIM d			9
SIM d × LIE b			4

pollen stainability (15%) than was observed in *L. alata* × *L. linearis* and the reciprocal hybrids of *L. alata* × *L. linifolia* (0–1%).

Morphology of the hybrids

In diploid species of sect. *Microcarpium*, the presence of petals appears to be dominant over the apetalous condition, judged from the consistent presence of petals in the petalous × apetalous hybrids. It is therefore of interest to examine this character in hybrids resulting from crosses between the petalous diploids and the apetalous polyploids. It was observed that all hybrids between diploids and tetraploids exhibited a variable number (0–4) of vestigial petals on different flowers of the same plant, whereas all hybrids between the diploids and hexaploids lacked petals completely.

When two of the diploid species *L. linearis* or *L. linifolia* were crossed to the tetraploid taxa, the resulting hybrids were somewhat intermediate in overall pubescence, leaf shape, capsule shape and size, sepal shape and size, and bracteole length. However, when *L. microcarpa*, the third diploid, was crossed with tetraploids, the F_1 hybrids were generally similar to their tetraploid parent in aspect but were diminutive in height and in leaf and flower size.

When all three of these diploids were each crossed to the hexaploid, *L. alata*, the resultant F_1 hybrids were more similar to *L. alata*, particularly in exhibiting its characteristic winged capsules. The hybrids *L. alata* × *L. linearis* and *L. alata* × *L. linifolia* resembled each other in their slightly narrower and longer capsules as compared with those of *L. alata*. Neverthelesss, *L. alata* × *L. linearis* had sparsely strigillose capsules, short sepals, and bracteoles shorter than the ovary, whereas *L. alata* × *L. linifolia* was completely glabrous, had elongate acuminate sepals, and had bracteoles longer than the ovary.

Hybrids between *L. microcarpa* and *L. alata* had small leaves and flowers, but were at least as robust and tall as *L. alata*. This is in sharp contrast to the situation in hybrids resulting from crosses between *L. microcarpa* and the tetraploid taxa (see above).

When diploid species (with pollen shed as tetrads) were crossed to hexaploid species (with pollen grains single), the resulting hybrids had single pollen grains only. Hybrids resulting from crosses between diploid species having single grains and tetraploid species having tetrad pollen showed a mixture of tetrad and single pollen in mature anthers. Crosses between diploids and tetraploids that both shed pollen as tetrads yielded hybrids that also produce tetrads. In the hybrid *L. linifolia* × *L. lanceolata*, however, where unreduced gametes of the diploid *L. linifolia* united with normal pollen of the tetraploid *L. lanceolata* to produce tetraploid hybrids, the pollen grains were shed mixed as tetrads and single grains. This result was not expected. In this hybrid, the morphology was intermediate between *L. linifolia* and *L. lanceolata*.

HYBRIDS BETWEEN THE DIPLOID GROUP AND THE *LUDWIGIA CURTISSII* COMPLEX

A summary of the crossing results is shown in Table 12. Some of these data are supplemented by study of natural hybrid populations, as is discussed below.

Meiotic analysis and pollen stainability

Six hybrid combinations were examined to determine percentage of stainable pollen. Two of these were also studied cytologically (Table 13).

TABLE 14. *Summary of crossing results between members of the* L. curtissii *complex and the* L. pilosa *group.*

L. curtissii as female parent	
CUR a × BRA	Only 4 seeds sown, none of which germinated.
CUR a × GLA d	Plants vigorous and floriferous.
CUR a × LAN a	Seeds failed to germinate.
CUR a × PIL b	Seeds failed to germinate.
CUR a × POL c	Plants flowered but were not vigorous.
CUR a × SPH b	Plants very vigorous and floriferous.
CUR a × SUF b	Plants dwarf, but were healthy and flowered.
L. curtissii as male parent	
ALA a × CUR a	Plants with reddish leaves, rosettelike, died when 6-leaved.
LAN a × CUR a	Plants started losing leaves when taller than 10 cm; set a few flowers.
PIL a × CUR f	Plants very vigorous and floriferous.
POL a × CUR a	Plants flowered but were not vigorous.
SPH a × CUR a	Plants very vigorous and floriferous.
L. simpsonii as female parent	
SIM a × ALA a	Plants vigorous and floriferous.
SIM c × LAN a	Seeds failed to germinate.
SIM a × PIL a	Died in cotyledon stage.
SIM a × SUF b	Seeds failed to germinate.
L. simpsonii as male parent	
ALA a × SIM a	Plants weak, leaves drooping, died when 6- or 8-leaved.
GLA b × SIM a	Plants vigorous and floriferous.
LAN a × SIM a	Plants with reddish leaves and much branched below; flowered recently.
TOR × SIM b	Seeds failed to germinate.

Of the 31 analyzable cells in *L. curtissii* (n = 32) × *L. linifolia* (n = 8), 22 formed strictly bivalents and univalents, ranging from (8–12)II + (24–16)I, with a modal configuration of 8II + 24I; nine cells formed multivalents, in which four cells had a configuration of 1III + (6–9)II + (25–19)I, and five cells showed 1IV + (6–9)II + (22–18)I. An exceptional cell with only 5II + 30I was also observed. In some cells chromosomes were so sticky that cytological analysis was impossible.

In *L. curtissii* × *L. microcarpa* (n = 8), only five analyzable cells were studied, the results being (14–15)II + (12–10)I. One or two sticky or precociously disjunct bivalents were noted in two cells.

Pollen stainability in all hybrids examined was very low, ranging 0–10% (Table 13). Despite this problem, a single F_2 individual of *L. curtissii* × *L. linifolia* was raised and another was raised from *L. curtissii* × *L. microcarpa*. The former remained sterile and died, whereas the latter died shortly after anthesis; it had 17% stainable pollen.

Morphology of the hybrids

Reciprocal hybrids of *L. curtissii* × *L. linearis* (n = 8) resemble each other. They are erect and branched on the upper stems. The tallest individual reached a height of 65 cm. The plants were extremely similar to *L. curtissii* in aspect, and before anthesis were thought to be selfed progeny of *L. curtissii*. In fact, even their flowers resembled those of *L. curtissii* except that their ovaries were slightly narrower and four-angled and their bracteoles were slightly shorter. Most flowers were apetalous; in a few cases one or two vestigial petals were present. After anthesis, the ovaries turned yellow and fell off. Pollen was shed as loose tetrads and single grains.

Hybrids between *Ludwigia simpsonii* (n = 24) and *L. linearis* could not be distinguished from *L. curtissii* × *L. linearis*.

Plants of *L. curtissii* × *L. linifolia* exhibited more morphological intermediacy than those of *L. curtissii* × *L. linearis*, although both resulted from hybridization between an octoploid and a diploid. Plants of *L. curtissii* × *L. linifolia* were smaller than either parent (about 25–30 cm high), much branched, and very floriferous (resembling *L. linifolia*). The flowers commonly had one to four petals (intermediate) and were congested at ends of branches (uncommon in both parents). The leaves were generally similar to those of *L. curtissii* in shape but were slightly smaller. The ovaries were about as long as those of *L. curtissii*, but were not accrescent and were narrower and four-angled. Sepals were intermediate in shape and size. Pollen was shed mostly as tetrads, and with some single grains as well. These hybrids did not usually set

TABLE 15. *Meiotic configurations and pollen stainability in hybrids resulting from crosses between members of the* L. curtissii *complex and the* L. pilosa *group.*

Hybrid Combination	Modal Meiotic Configuration Observed	Number of Cells with Modal Configuration/ Total Number of Cells Examined	Pollen Stainability (%)
L. curtissii as one parent			
CUR a × GLA d			5
CUR a × POL c	1III + 4II + 37I	1/1	32
CUR a × SPH b			31
CUR a × SUF b	(11–13)II + (26–22)I	5/5	9
LAN a × CUR f			4
PIL a × CUR a	1III + (10–13)II + (25–19)I	4/16	6
POL a × CUR a	chromosomes very sticky, not analyzable		25
SPH a × CUR f	(1IV) + (1–3)III + (6–13)II + (14–30)I	6/6	17
L. simpsonii as one parent			
GLA b × SIM a			3
LAN a × SIM a	1III + 5II + 27I	1/3	11
SIM a × ALA a			20

seed, although one or two viable seeds were occasionally obtained.

Plants of *L. curtissii* × *L. microcarpa* were very vigorous and floriferous, up to 80 cm high, and much branched. The leaves were similar to those of *L. curtissii*, but smaller. Similarly, all floral parts were reduced and somewhat resembled those of *L. microcarpa*. In the hybrid, however, the nectary discs were always distinctly raised, unlike those of *L. microcarpa*, in which they were nearly flat. The ovaries usually shriveled after anthesis, although in exceptional cases one to three seeds were produced.

Plants of *L. microcarpa* × *L. simpsonii* were also very vigorous. Their floral parts were similar to those of *L. curtissii* × *L. microcarpa*, and their flowers were sterile. These hybrids were smaller (up to 50 cm high) and had slightly broader leaves. As plant height and leaf shape are somewhat variable characters, it is difficult to distinguish between these two hybrids when they occur together in nature.

HYBRIDS BETWEEN THE *LUDWIGIA CURTISSII* COMPLEX AND THE *L. PILOSA* GROUP

As with crosses between the diploid group and the *L. pilosa* group, these crossing results were quite variable, ranging from total failure to germinate to vigorous and floriferous F_1 hybrids (Table 14). None of these F_1 plants were observed to set any seed, however, while most of the hybrids resulted from crosses between the *L. curtisssii* complex and the *L. pilosa* group set at least a few viable seeds. An F_2 family of 12 vigorous plants of *L. simpsonii* × *L. alata* was established.

Meiotic analysis and pollen stainability

In hybrids with *L. simpsonii* as one of the parents, meiosis was studied only in *L. lanceolata* × *L. simpsonii* (Table 15). Only three metaphase I cells were obtained, which had three to five bivalents, most of which were chromosomes connected by a chromatin thread and were aligned randomly. A trivalent was seen in one of the cells.

In hybrids with *L. curtissii* as the male parent, *L. polycarpa* × *L. curtissii* had sticky chromosomes, which rendered study of meiosis difficult.

Four other hybrid combinations were studied cytologically. In *L. curtissii* × *L. polycarpa*, only one first metaphase cell was analyzable; it showed a configuration of 1III + 4II + 37I with two attenuated bivalents in the equatorial plate.

The other three hybrids resulting from crossing *L. curtissii* and the tetraploid species showed significantly higher chromosome associations. A maximum of 13 bivalents were seen in at least some of the cells, and one tetravalent and a maximum of three trivalents were observed in others (Table 15). The meiotic metaphase figures of these hybrids generally consisted of bivalents or multivalents

aligned in the equatorial plate with many univalents scattered throughout the cell. The following cytological aberrations were occasionally observed: attenuated bivalents, precociously separated bivalents, attached univalents, and sticky chromosomes.

The pollen stainability was, surprisingly, higher in *Ludwigia curtisii* × *L. polycarpa* (32%), which showed the least chromosome associations, and in *L. polycarpa* × *L. curtissii* (25%), which had stickier chromosomes than in other hybrids known to have higher levels of chromosome pairing. Since in *L. curtissii* × *L. polycarpa* only one meiotic cell was studied, the observed configuration possibly could represent the lower limit of chromosome association in this hybrid. Variability of chromosome pairing is a common phenomenon in species hybrids. The stickiness of meiotic chromosomes of *L. polycarpa* × *L. curtissii*, however, may have a genetic basis, as it was rarely shown by either of the parental species, or the stickiness might be attributable to environmental factors. My unpublished study of meiosis in a sterile natural hybrid between *L. spathulata* and *L. palustris* (both sect. *Dantia*) was not successful in 1979 due to pronounced chromosome stickiness. Nevertheless, very clear chromosomal configurations for the same clone were obtained the next year.

In spite of the prevalence of univalents in meiosis, these hybrids usually set at least a few seeds. They were quite unlike the hybrids between the *L. curtissii* complex and the diploid group, which were completely sterile. This difference is probably attributable to the fact that, when both parents are polyploids, the development of functional pollen and ovules is better able to withstand the random segregation or loss of some chromosomes (as lagging univalents) in meiosis because of genetic redundancy.

Morphology of the hybrids

The hybrids were generally intermediate morphologically. The leaves were characteristically oblanceolate or narrowly oblanceolate. The intermediate nature of the size, shape, and pubesence of the capsules could be diagnostic, although the winged capsules characteristic of *L. alata* and *L. lanceolata* were not taxonomically useful, as the capsules of all the hybrids shriveled to some extent and thus appeared winged. The differences between hybrids with *L. curtissii* and with *L. simpsonii* as one parent were strictly quantitative, with the latter being slightly smaller in their floral features and occasionally in height also. These differences were obvious only when the two hybrids were brought together and compared. When *L. curtissii* and *L. simpsonii*, both of which shed pollen singly, were crossed with species in the *L. pilosa* group, which shed pollen as tetrads, the resultant hybrids consistently produced a mixture of pollen in loose tetrads and single grains.

Ecology and Geographical Distribution

With the exceptions of *L. stricta*, which is endemic to Cuba, and *L. polycarpa*, which is distributed mainly in the north-central United States, sect. *Microcarpium* is confined primarily to the Coastal Plain of the United States (Fig. 20). The detailed geographical distribution of each taxon in *Ludwigia* sect. *Microcarpium* is presented in a companion taxonomic paper (Peng, in press).

The Coastal Plain is defined geologically as the flat area between the Atlantic and Gulf coasts and the Piedmont, and extending from the Gulf of Mexico to southern New England (Peattie, 1922). The soils of this area are chiefly gray sands and sandy loams, except in the swamps where the prevailing sands are covered by muck or peat (Cooke, 1925). On the Coastal Plain, especially in areas close to the coast, the water table is seldom very far below the surface, and many areas are periodically or permanently flooded (Gleason & Cronquist, 1964). At one end of the Coastal Plain, in southern Texas, the climate is semiarid and the soils are alkaline, containing a high proportion of clay (Hunt, 1974).

In addition to this general distribution of species of sect. *Microcarpium* in North America, several species extend further south. *Ludwigia alata* occurs in Jamaica, *L. simpsonii* in Cuba and Jamaica, and *L. curtissii* in the Bahamas. *L. linifolia* is disjunct to Tabasco, in the Yucatán Peninsula of Mexico, and *L. microcarpa* ranges to the Bahamas, Cuba, and Jamaica. *Ludwigia stricta*, endemic to Cuba, is the only species of the section that does not occur in the United States.

Like *Ludwigia* species occurring in other parts of the world, plants of sect. *Microcarpium* grow in at least seasonally wet habitats. They are commonly found along alluvial ground or in the shallow water of many areas, including ponds, lakes, rivers, streams, lagoons, sloughs, backwaters, swales, wet meadows or prairies, open swamp forests, drainages, and irrigation ditches. All species grow in sandy or occasionally peaty soils.

Sympatric Occurrence and Natural Hybridization

The results of experimental hybridizations reveal that vigorous and floriferous hybrid individuals can

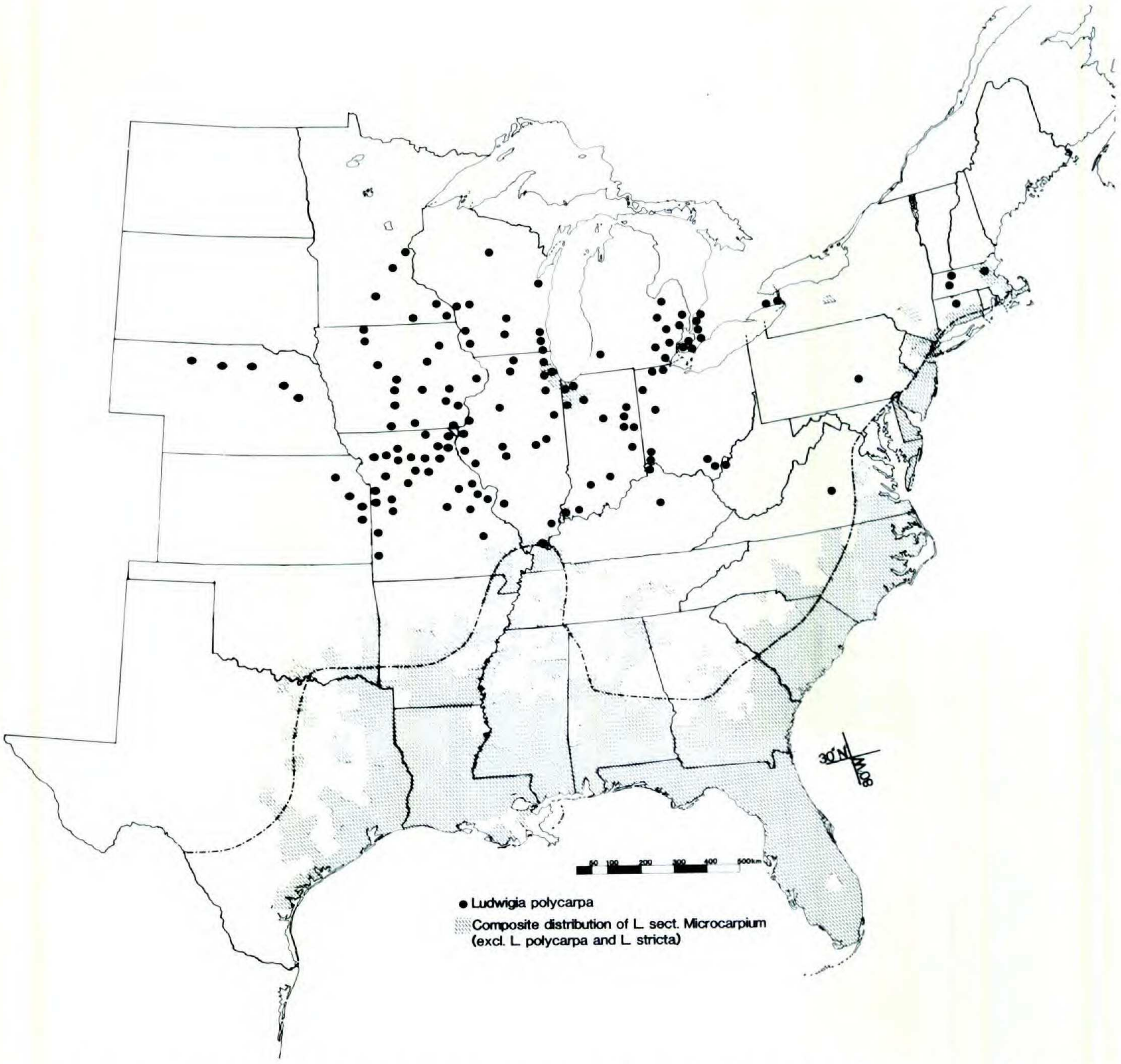

FIGURE 20. Distribution of *Ludwigia* sect. *Microcarpium* in North America, with shading to indicate the combined distribution of all species of this section except *L. polycarpa* and *L. stricta*. Distribution of *L. polycarpa* is indicated by dots; *L. stricta* is endemic to Cuba. Boundary of the Coastal Plain in the United States is marked by dashed line. Other occurrences of several species in the Bahamas, Cuba, Jamaica, and Mexico, and a disjunct population of *L. polycarpa* in Kootenal Co., Idaho, are not mapped.

readily be obtained between most members of sect. *Microcarpium*. Exceptions involved some crosses between members of the *L. pilosa* group (the tetraploid taxa plus the hexaploid *L. alata*) and either the diploid species (*L. linearis*, *L. linifolia*, and *L. microcarpa*) or members of the *L. curtissii* complex (*L. curtissii* and *L. simpsonii*) (Fig. 5). These crosses resulted in seeds that failed to germinate or in inviable hybrids. Even in these instances, if alternative parental strains were utilized to vary the genetic composition of the hybrids, vigorous F_1 individuals could sometimes be obtained. The general lack of postzygotic barriers, in conjunction with the facts that most *Ludwigia* species have overlapping geographic ranges, similar habitat requirements, and similar flowering periods (during the summer), and that they are at least facultatively outcrossing, favor natural hybridization.

Field observations and examination of herbarium specimens suggest that natural interspecific hybridization involving species of sect. *Microcarpium* occurs frequently. Intersectional hybridization is also quite common; at least seven hybrid combinations bridging sect. *Microcarpium* and sect. *Dantia* have been observed.

Observation of individuals exhibiting a combination of characters intermediate between distinct taxa initially suggests the possibility of natural hybridization. However, the members of two species

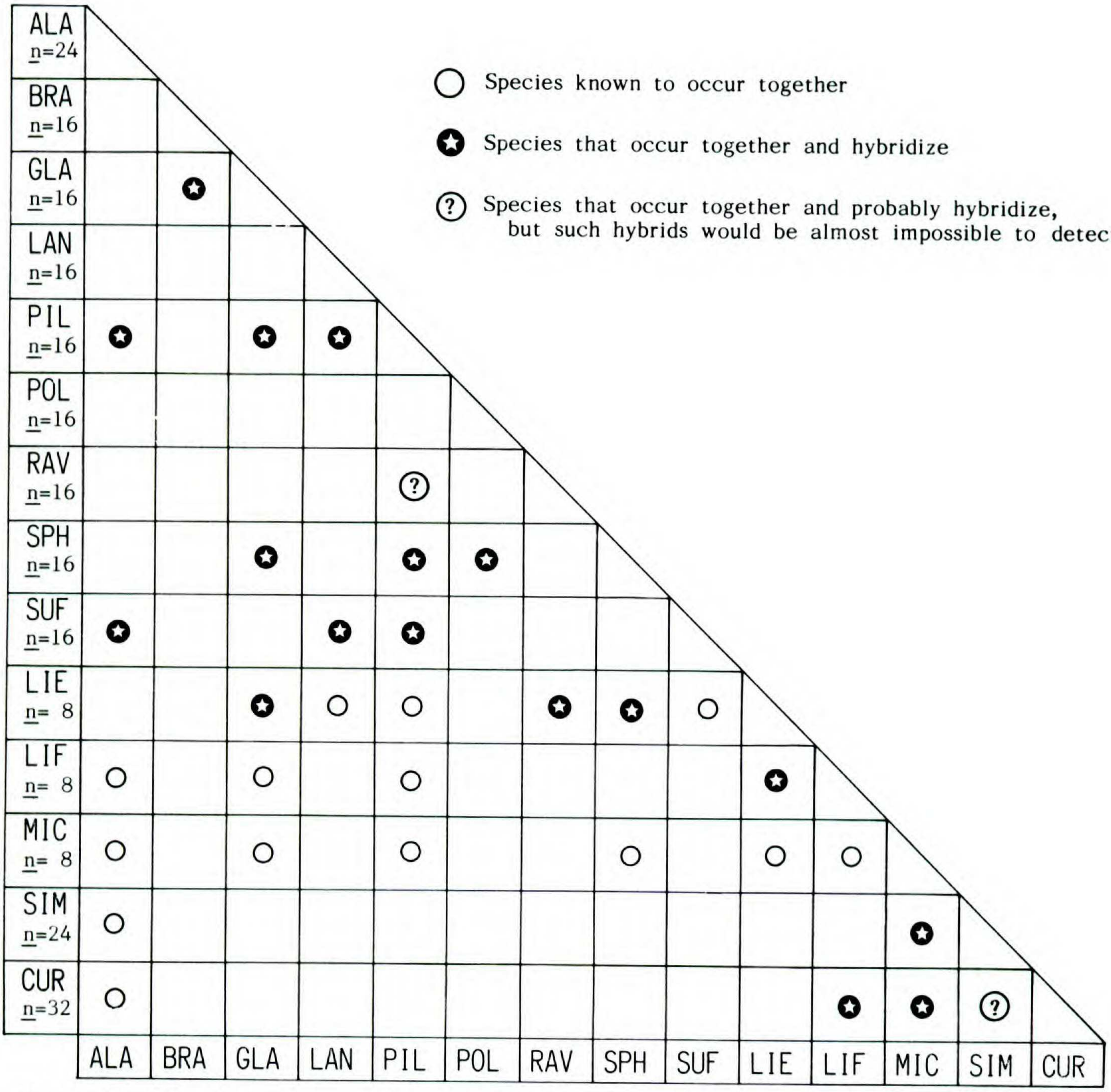

FIGURE 21. Sympatric occurrence of species in *Ludwigia* sect. *Microcarpium*. Acronyms are those given in Table 3.

pairs, *L. alata* ($n = 24$)/*L. lanceolata* ($n = 16$) and *L. curtissii* ($n = 32$)/*L. simpsonii* ($n = 24$), are usually so similar in appearance (especially as herbarium specimens) that their hybrids cannot be recognized readily.

As indicated earlier, capsule shape and size are the most important characters for detecting hybrids in sect. *Microcarpium*. Other useful features include overall pubescence, seed-surface cell shape and orientation, presence or absence of petals, whether pollen grains are shed singly or in tetrads, and pollen stainability. Seed-surface pattern is useful only for hybrids within the tetraploid group (including *L. alata*), as these nine taxa are interfertile, yield abundant seeds, and are diverse with respect to this character. Absence of developing fruits and low levels of pollen stainability are characteristic of hybrids resulting from all other interploid crosses, with the exception of *L. curtissii* ($n = 32$) × *L. simpsonii* ($n = 24$). Chromosome number and meiotic chromosome behavior are useful indicators of natural hybrids when the parents involved have different chromosome numbers or differentiated genomes.

Field experience indicates that the occurrence of natural hybrid populations is fairly common wherever two or more species grow together. Hybrids resulting from crosses between members of the *Ludwigia pilosa* group are very common. Also, many hybrid combinations have been observed in-

volving the following species: *L. microcarpa*, *L. curtissii*, *L. simpsonii*, *L. palustris* ($n = 8$; sect. *Dantia*), and *L. repens* ($n = 24$; sect. *Dantia*).

Anderson (1948) suggested that disturbed habitats often afford conditions suitable for establishment of natural hybrids. This does not seem to be the case for *Ludwigia* hybrids, since the parental species themselves nearly always grow with the hybrids. Figure 21 illustrates the sympatric occurrence and known cases of hybridization in nature for all taxa of sect. *Microcarpium* except the Cuban endemic *L. stricta*. Sympatry was determined primarily from personal observation (in 1979, 1980, 1982) and supplemented by field notes of Peter H. Raven and from mixed herbarium collections. Voucher information for the suspected natural hybrids is presented below with comments when appropriate. Chromosome numbers of the parents are indicated in parentheses after their names. The sequence of epithets in each formula is alphabetical.

HYBRIDS INVOLVING MEMBERS OF *LUDWIGIA* SECT. *MICROCARPIUM*

Ludwigia alata ($n = 24$) × **L. pilosa** ($n = 16$). U.S.A. FLORIDA: Franklin Co., 38.8 mi. W of jct. US 98 and US 319, *Peng 4346* (MO). Wakulla Co., 5 mi. S of Sopchoppy on US 319, *Lazor 4984* (FSU, VDB); 1 mi. S of Sopchoppy on US 319, *Morar 29* (FLAS, GH, MSU, USF). Walton Co., Freeport, *Godfrey 57653* (FSU). GEORGIA: Charlton Co., of Folkston, Okefenokee Swamp, Camp Cornelia, *Jones 22996* (GA). MISSISSIPPI: Hancock Co., along Jordan River S of Kiln, *Jones 9539* (MISS).

Notes. *Peng 4346* from Franklin Co., Florida, was found in a very wide roadside drainage ditch (ca. 10–15 m across) at the edge of a pine forest. Hybrids were very abundant and mixed with a large population of *L. alata* and a few individuals of *L. pilosa*. Large populations of *L. linifolia* and *L. microcarpa* were also present. This hybrid exhibited meiotic configurations of 15–16 bivalents and 10–8 univalents. It had 84% stainable pollen.

Ludwigia alata ($n = 24$) × **L. suffruticosa** ($n = 16$). U.S.A. FLORIDA: Hillsborough Co., on E side of FL 581, 2.8 mi. N of FL 582, *Peng 4329* (MO). Lake Co., Tavares, *Biltmore Herbarium 4170*[d] (DS); vicinity of Eustis, *Hitchcock in 1894* (F), *Nash 1154* (NY); 5 mi. SE of Lebanon Station, *Kral 7807* (GA, GH, NCU, US, USF). Taylor Co., N edge of US 27 at Fenholloway River Bridge, *Nelson 667* (USCH); ca. 20 mi. NW of Cross City, *Godfrey & Houk 60296* (FSU, MSU, NCU, SMU).

Notes. More or less congested inflorescence and winged capsules are characteristic of this hybrid combination. The hybrids were often identified as *L. alata* when they had lax inflorescences. In these cases, however, reduced levels of stainable pollen and intermediate seed surface cell pattern were useful in revealing the hybrids. *Peng 4329* showed a meiotic configuration of 15 bivalents and 10 univalents.

Ludwigia curtissii ($n = 32$) × **L. linifolia** ($n = 8$). U.S.A. FLORIDA: Monroe Co., Pine Crest, *Moldenke 856*[a] (MO, NY). Pasco Co., 1 mi. E of Gowers Corner off US 41, *Ray et al. 9932* (USF).

Notes. This hybrid is very similar to *L. curtissii* in aspect, but its pollen is not stainable, and its ovaries abort. One putative parent, *L. linifolia* (*Ray et al. 9934*, USF), was collected at the same locality as the hybrid (*Ray et al. 9932*).

Ludwigia curtissii ($n = 32$) × **L. microcarpa** ($n = 8$). U.S.A. FLORIDA: Martin Co., 4.3 mi. E of Okeechobee and Martin Co. line, on FL 710, at Brady Ranch, *Peng 4202* (MO).

Notes. The hybrid population was found in a wide swampy depression between the highway and a railroad, growing with both putative parents. The hybrid had a single analyzable metaphase I cell which showed 8 bivalents and 24 univalents. Pollen stainability was 16%.

Ludwigia glandulosa subsp. **glandulosa** ($n = 16$) × **L. pilosa** ($n = 16$). U.S.A. ALABAMA: Covington Co., along Co. Rd. 42, 15 mi. E of Brooklyn, *Kral 40992* (FLAS, GH, MO, NCU, NY, US, USF, VDB). GEORGIA: Grady Co., 13 air mi. SW of Cairo, 5 air mi. NE of Concord, Florida, with *L. pilosa*, *Anderson 4044* (MO, FSU). MISSISSIPPI: Jackson Co., Ocean Springs, *Demaree 32174* (RSA, SMU); on MS 90, 2 mi. W of US 10 and MS 90, with *L. pilosa*, *Peng 4354-A* (MO). NORTH CAROLINA: Hyde Co., 1.1 mi. N of Scranton Creek on US 264, with both putative parents, *Duke 54-232B* (NCU); 1.2 mi. N of Scranton Creek on US 264, with both putative parents, *Duke 54-276*, *54-277*, and *54-278* (NCU).

Ludwigia glandulosa subsp. **glandulosa** (n = 16) × **L. linearis** (n = 8). U.S.A. GEORGIA: Long Co., 4.2 mi. SW of jct. of US 301 and 25, and GA 99, on US 301 and 25, *Peng 4118* (MO). NORTH CAROLINA: Craven Co., 0.8 mi. N of US 17 on Co. Rd. 1224 (road to Tuscarora), *Boufford et al. 21443* (MO), *Peng 3740* (MO).

Notes. Both hybrid populations were found along with the putative parents. *Peng 3740* from Craven Co., North Carolina, was found in a wet drainage ditch about 60–80 cm wide. The population consisted of ca. 15–20 floriferous individuals, some of which were even more robust than the putative parents, which grew next to and on either side of the hybrid population. The modal meiotic configuration of this hybrid was 3–5 bivalents and 18–14 univalents. Heteromorphic pairs were sometimes observed. A trivalent was seen in one of the 26 cells studied. The pollen stainability was 2%. *Peng 4118*, from Long Co., Georgia, consisted of a few scattered individuals. The putative parents were growing nearby on a grassy roadside shoulder.

Ludwigia glandulosa subsp. **glandulosa** (n = 16) × *L. sphaerocarpa* (n = 16). U.S.A. MISSOURI: Butler Co., swamps, *Eggert in 1893* (MO). SOUTH CAROLINA: Clarendon Co., near shore of Lake Marion, ca. 4.5 mi. SW of St. Paul off US 15, *Bradley & Sears 3561* (BOON, East Carolina Univ., NCU, WCUH).

Notes. *Bradley & Sears 3561* is a mixed collection; all four specimens contain a mixture of hybrids and individuals of *L. glandulosa* subsp. *glandulosa*. Although evident hybrids between *L. glandulosa* subsp. *glandulosa* and *L. sphaerocarpa* were found in Butler Co., Missouri, specimens of *L. sphaerocarpa* have not yet been collected from that state.

Ludwigia lanceolata (n = 16) × **L. pilosa** (n = 16) [*Ludwigia* ×*simulata* Small]. U.S.A. FLORIDA: Franklin Co., Apalachicola, *Chapman s.n.* (F, US). Highlands Co., Bear Point, Lake Childs, *Brass 15532* (GH, US). West Florida, *Biltmore Herbarium* (NY, holotype of *Ludwigia* ×*simulata* Small).

Notes. The holotype of *L.* ×*simulata* is characterized by being densely strigillose throughout and having four-angled or slightly winged capsules, isodiametric seed-surface cells, and pollen shed in tight tetrads. Such a combination of characters clearly suggests *L. lanceolata* (which is glabrous, has winged capsules, isodiametric seed surface cells, and pollen shed in tetrads) and *L. pilosa* (which is densely hirsute, has rounded capsules, isodiametric seed-surface cells, and pollen shed in tetrads) as putative parents. Furthermore, *L.* ×*simulata* is comparable to the experimental hybrids obtained from reciprocal crosses between those species.

Ludwigia lanceolata (n = 16) × **L. suffruticosa** (n = 16). U.S.A. FLORIDA: Charlton Co., Okefenokee Swamp, *Harper 1483* (GH, MO, NCU, NY, US). Hillsborough Co., 1.5–1.7 mi. S of FL 674, on E side of Taylor Gill Dr., *Peng 4324, 4328* (MO).

Notes. Both putative parents as well as *L. linearis* were present in the same drainage ditch where *Peng 4324* and *Peng 4328* were collected. *Peng 4324* showed 16 bivalents in diakinesis and metaphase I cells. One precociously separating bivalent was occasionally observed. *Peng 4324* had 85% stainable pollen.

Ludwigia linearis (n = 8) × **L. linifolia** (n = 8). U.S.A. FLORIDA: Palm Beach Co., along the S side of Co. Rd. 74, 1.5 mi. W of the Turnpike, Palm Beach Gardens, *Popenoe 1957* (MO).

Notes. This plant is only 25–30 cm tall and has crowded leaves. It showed features somewhat intermediate between those of *L. linearis* and those of *L. linifolia* in its flowers and capsules. The pollen was shed as tetrads, many of which are unstainable. This plant probably is a hybrid between *L. linearis* and *L. linifolia*, although the experimental hybrids obtained between these taxa have been more robust.

Ludwigia linearis (n = 8) × **L. sphaerocarpa** (n = 16). U.S.A. ALABAMA: Covington Co., Conecuh National Forest, SW Andalusia, *Kral 44732* (ENCB, SMU, VDB).

Notes. The plants are very vigorous and floriferous. One to four vestigial petals are present in most flowers. Seed set is very low or possibly nonexistent.

Ludwigia microcarpa (n = 8) × **L. simpsonii** (n = 24). U.S.A. FLORIDA: Charlotte Co., Punta Gorda City, on US 41, ca. 1 mi. S of jct. of US 17 and 41, *Peng 4297* (MO). Clay Co., 5 mi. W of Penny Farms on FL 16, *Peng 4160* (MO). Collier Co., 4.8 mi. W of Monroe Station, on N side of US 41, *Peng 4263* (MO).

Sumter Co., Cedar Hammock, 1894, *Lewton s.n.* (NY).

Notes. *Peng 4297* from Charlotte Co., Florida, was found intermixed with both parents. Also occurring here were *L. microcarpa*, *L. repens* (sect. *Dantia*), and the intersectional hybrid between them. Of the nine meiotic metaphase I cells examined from *Peng 4297*, seven showed 8 bivalents and 16 univalents, and two showed 9 bivalents and 14 univalents. In the latter, one of the bivalents separated precociously. Pollen stainability was 4%. *Peng 4160*, from Clay Co., Florida, was collected from a large population located in a waterlogged roadside ditch along the margin of a pine woodland. The hybrids were intermixed with one of the putative parents, *L. microcarpa*. Across the highway in the similar habitat was another very large, pure population of *L. microcarpa*. *Peng 4160* had 9% stainable pollen. *Peng 4263*, from Collier Co., Florida, was found in an open palmetto–cypress forest, with both putative parents growing nearby. One somewhat analyzable metaphase I cell from this plant showed 8 bivalents and 16 univalents. Pollen stainability was 24%.

Ludwigia pilosa ($n = 16$) × **L. sphaerocarpa** ($n = 16$).

Before discussing this hybrid combination, a few comments on *L. sphaerocarpa* are appropriate. This species is quite variable and has a widely scattered distribution (Fig. 22). Populations of *L. sphaerocarpa* consist of individuals that are extremely varied in overall pubescence, leaf shape and size, fruit size, and density of fruits on branches. Three varieties (var. *jungens*, var. *macrocarpa*, and var. *deamii*) have been recognized previously within this species (Fernald & Griscom, 1935) based on various combinations of the above characters. Study of numerous herbarium specimens not available to Fernald & Griscom, however, revealed that correlations between these characteristics are not consistent. It is of interest to note that, although seed-surface cell pattern is generally very regular within populations of members of sect. *Microcarpium* (Figs. 1–3), this is not the case for *L. sphaerocarpa* (Fig. 4). The seed surfaces are arranged in columnar cells both transversely elongate and parallel to the seed length, with the former alignment often predominant in the central part of the seeds (Fig. 4). Seeds with variously oriented surface cells are also seen in some populations. A comparison of the irregular seed surface pattern in *L. sphaerocarpa* with that of various artificial hybrid combinations (Figs. 6–19) strongly suggests that earlier hybridizations within the interfertile tetraploid group of sect. *Microcarpium* may have resulted in production of this widespread series of populations that have more or less stabilized in some of their characteristics.

Morphological variation in *L. sphaerocarpa* is further complicated by its frequent natural hybridization with *L. pilosa* (and perhaps with *L. ravenii* as well, although it would be difficult to distinguish these hybrids from those involving *L. pilosa*), which has apparently resulted in many hybrid swarms or introgressed populations. These plants are generally neither typical of *L. pilosa* nor of *L. sphaerocarpa* and exhibit varying degrees of intermediacy between the two species. The diagnostic characters for the hybrids include overall pubescence, leaf shape, bracteole size and location, sepal shape and size, and color of abaxial leaf venation. Examples of populations of such intermediates are too numerous to cite here. Instead they have been mapped (Fig. 22). It is of interest to note that some of the hybrid populations occur in central and southern Florida where typical *L. pilosa* and *L. sphaerocarpa* are absent; this suggests that physiological characteristics, and thereby ecological tolerances, may recombine into novel adaptive combinations in the hybrids also.

Artificial hybrids between *L. pilosa* and *L. sphaerocarpa* were synthesized in an experimental greenhouse. Plants of this hybrid combination showed 15–16 bivalents and exhibited the highest level of stainable pollen (98%) among all the tetraploid hybrids.

Ludwigia pilosa ($n = 16$) × **L. suffruticosa** ($n = 16$) [*L. capitata β pubens* Torrey & A. Gray]. U.S.A. FLORIDA: Citrus Co., 5 mi. S of Homosassa, *Kral 7771* (FLAS, GH, both mixed with *L. suffruticosa*). Gadsden Co., along Old Bainbridge Rd. (Rte. 173); 0.5 mi. NW of Ochlockonee River bridge, NW of Tallahassee, *Anderson 7486* (FSU). Seminole Co., W shore of Prairie Lake, *Schallert 16009, 28652* (S). GEORGIA: McIntosh Co., on Sapelo Isl., ca. 1.4 mi. WNW of S tip of Blackbeard Isl., *Duncan 20445* (DUKE, F, GH, NCU, SMU, TEX, US, USF). Wayne Co., near Jessup, *Biltmore Herbarium 4174*[c] (US). GEORGIA (?): *Baldwin Herbarium* (NY, holotype of *Ludwigia capitata* Michaux *β pubens* Torrey & A. Gray, mixed with *L. suffruticosa* Walter). SOUTH CAROLINA: Darlington Co., Hartsville, *Smith 44* (NCU). Georgetown Co., North Santee, *Radford 28678* (NCU, NY, VDB).

FIGURE 22. Distribution of *Ludwigia pilosa* (shading), *L. sphaerocarpa* (stippling), and their natural hybrids (dots).

Ludwigia polycarpa ($n = 16$) × **L. sphaerocarpa** ($n = 16$). U.S.A. INDIANA: Starke Co., border of Bass Lake, 5 mi. S of Knox, *Kriebel 5715* (SMU); SW corner of Bass Lake, *Friesner 16306* (CAS, NY).

Notes. *Ludwigia polycarpa*, which occurs in the central Midwest (Fig. 20), is effectively isolated geographically from all other species of sect. *Microcarpium*, with the exception of *L. sphaerocarpa*. Where the two species come into contact, hybridization occurs.

The following is a list of collections of hybrids for which the identity of the putative parents is not certain:

?Ludwigia linearis ($n = 8$) × **L. ravenii** ($n = 16$). U.S.A. NORTH CAROLINA: Duplin Co., 3 mi. E of Sarecta, *Beal 3674* (NCSC).

Notes. This is a much-branched, densely villous plant with sublinear leaves and small, elongate-pyramidal ovaries that abort after anthesis. The pubescence suggests that either *L. pilosa* or *L. ravenii* is involved in the parentage. The aborted ovaries indicate that hybridization involved one of the above species and a taxon outside of the interfertile *L. pilosa* group. The elongate ovaries and narrow leaves indicate clearly that either *L. linearis* or *L. linifolia* is the other parent. Floral characters as well as geographical distribution of these species, however, indicate that *Beal 3674*

from Duplin Co., North Carolina, is probably a hybrid between *L. linearis* and *L. ravenii*.

Ludwigia microcarpa ($n = 8$) × **L. curtissii** ($n = 32$)/**L. simpsonii** ($n = 24$). U.S.A. FLORIDA: Flagler Co., 6 mi. E of Co. line, Hwy. 28 near Andalusia, *West in 1940* (FLAS). Lake Co., 7 mi. SW Okahumpka, *Kral 7611* (FLAS, FSU, GH).

Notes. *Kral 7611* represents a mixture of several species and hybrids. Specimens deposited in GA, GH, US, VDB are *L. microcarpa*, whereas the specimen at FSU contains both the hybrid and either *L. curtissii* or *L. simpsonii*, the separation of which is difficult, since mature capsules are not present.

?Ludwigia pilosa ($n = 16$) × **L. suffruticosa** ($n = 16$). U.S.A. FLORIDA: Jackson Co., Ocheesee Lodge Landing S of US 90, near Sneads, *Jones 23569* (GA). Co. unknown, S Florida, *Chapman Herbarium* (NY). GEORGIA: Lee Co., near US 19, ca. 4 mi. S of Smithville, *Moran 2551* (GA).

Notes. These specimens are less pubescent than typical *L. pilosa* × *L. suffruticosa* hybrids. They are for the most part densely strigillose only in the branched and somewhat lax inflorescence and may represent backcrossed populations or segregates of advanced generation of the hybrid *L. pilosa* × *L. suffruticosa*.

HYBRIDS BETWEEN MEMBERS OF *LUDWIGIA* SECTS. *MICROCARPIUM* AND *DANTIA*

Hybrids between members of sects. *Microcarpium* and *Dantia* are easy to recognize; members of sect. *Microcarpium* are erect plants with alternate leaves, whereas plants belonging to sect. *Dantia* are prostrate and have opposite leaves. Intermediacy in these two characters signals hybridization between the two sections.

Ludwigia arcuata ($n = 16$; sect. *Dantia*) × **L. pilosa** ($n = 16$). U.S.A. ALABAMA: Mobile Co., Audubon Bird Sanctuary, Dauphin Isl., *Deramus D643* (DS, UNA).

Notes. Hybrids of this combination were synthesized in the experimental greenhouse. They showed a modal meiotic configuration of 12–15 bivalents and 8–2 univalents; 1–2 trivalents were sometimes observed. The high degree of chromosome pairing observed between species of the two sections was quite unexpected. This artificial hybrid, however, had only 38% stainable pollen. Since selfing in the hybrid is physically impossible, as in *L. pilosa*, artificial pollination was attempted in order to investigate seed set. None of the pollination attempts yielded any seed set, even though some seeds were observed in mature capsules of the natural hybrids.

Ludwigia curtissii ($n = 32$) × **L. repens** ($n = 24$; sect. *Dantia*). U.S.A. FLORIDA: Glades Co., 4.4 mi. SE of jct. of FL 29 with US 27, with both parents, *Raven 18680* (MO). Lee Co., on US 41, 5 mi. N of Ft. Meyers, with both parents, *Dille & Dille 379* (MO).

Notes. *Ludwigia curtissii* ($n = 32$) and *L. simpsonii* ($n = 24$) hybridize with *L. repens* in nature. Unless chromosome numbers are counted, it is unlikely that one would be able to distinguish between these two hybrid combinations morphologically.

Ludwigia curtissii ($n = 32$)/**L. simpsonii** ($n = 24$) × **L. repens** ($n = 24$; sect. *Dantia*). U.S.A. FLORIDA: Charlotte Co., 12 mi. S of Punta Gorda, *Kral 18058* (VDB). Lee Co., 5 mi. S of Bonita Springs, *Crevasse in 1940* (FLAS). Manatee Co., Bradentown, *Cuthbert in 1926* (FLAS). Wakulla Co., between Hwys. 365–367, N of Spring Creek, *Lazor 4561* (NCU).

Ludwigia repens ($n = 24$; sect. *Dantia*) × **L. simpsonii** ($n = 24$). U.S.A. FLORIDA: Charlotte Co., Punta Gorda City, on US 41, ca. 1 mi. S of jct. of US 17 and 41, *Peng 4296* (MO).

Notes. This hybrid grew intermixed with both putative parents along a roadside field. It formed 0–1 bivalent(s) and 48–46 univalents in metaphase I cells. This result corroborates an earlier report by Schmidt (1967) [FLORIDA: Glades Co., 8.4 mi. SE of jct. of FL 29 on US 27, *Raven 18849* (DS)].

Ludwigia glandulosa subsp. **glandulosa** ($n = 16$) × **L. palustris** ($n = 8$; sect. *Dantia*). U.S.A. ARKANSAS: Clark Co., Okolona, *Demaree 16120* (DS, GA, GH, MO, NY, SMU, TENN). NORTH CAROLINA: Co. unknown, stagnant water just S of Upper Littel River on US 401, *Lloyd in 1962* (MO). Johnston Co., 4.9 mi. W of NC 210 and US 70, on US 70, E of Clinton, *Ahles 59736* (NCU), *61803* (DS,

NCU, SMU). OKLAHOMA: Johnston Co., Devil's Den, 4.6 mi. NW of Tishomingo, *Crutchfield 2882* (LL). VIRGINIA: Fluvanna Co., just S of Rt. 696, 1 mi. S of Rt. 250, *Diggs & Diggs 353* (NCU).

Notes. Plants of *Lloyd in 1962* from North Carolina showed 24 univalents in meiosis (Raven, pers. comm.). Schmidt (1967) reported "at most three weakly joined bivalents" in the hybrid plant from North Carolina [Harnet Co., 5.1 mi. S of Lillington, *Lloyd 1022* (DS)]. Artificial hybrids recently synthesized, however, showed higher chromosome associations in meiosis, in the range five to eight bivalents modally.

Ludwigia microcarpa ($n = 8$) × **L. palustris** ($n = 8$; sect *Dantia*). U.S.A. FLORIDA: Franklin Co., 41.7 mi. W of jct. of US 98 and 319, with both putative parents, *Peng 4349* (MO). Hamilton Co., off I-75, ca. 1 mi. N of Columbia Co. line, *Bowers & Wofford 71-550-F* (TENN, a mixture of *L. repens*, *L. microcarpa*, and *L. microcarpa* × *L. palustris*). Lake Co., 3.7 mi. S of Mascotte city limit, on Co. Rd. 33, with *L. microcarpa* and *L. palustris*, *Peng 4167* (MO). GEORGIA: Camden Co., 6.6 mi. S of Woodbine on US 17, with *L. microcarpa* and *L. repens*, *Raven 18704* (MO). NORTH CAROLINA: Jones Co., near NC 41, 0.7 mi. E of Taylor's Corner, *Radford 37152* (NCU).

Notes. *Peng 4349* from Franklin Co., Florida, was collected from a large population growing intermixed with both putative parents along a swampy ditch. *Peng 4167* from Lake Co., Florida, was found growing with both putative parents in a narrow ditch. Pollen stainability was 5% in *Peng 4349*. Artificial hybrids of this combination were synthesized and showed 2–5 bivalents and 12–6 univalents in meiotic metaphase I.

Observations from experimental hybridization indicate that hybrid combinations of *L. microcarpa* ($n = 8$) × *L. palustris* ($n = 8$; sect. *Dantia*) and *L. microcarpa* ($n = 8$) × *L. repens* ($n = 24$; sect. *Dantia*) are prostrate herbs with similar leaf shape and minute flowers that abort after anthesis. In *L. microcarpa* × *L. palustris*, the flowers are apetalous as in both parents, and the phyllotaxy is intermediate; the plants have alternate, opposite, and subopposite leaves. By contrast, in *L. microcarpa* × *L. repens* the leaves are always opposite as in *L. repens*, and some of the flowers have one to four vestigial petals. The apparent dominance of the traits of the hexaploid parent over those of the diploid parent is probably due to a multiple dosage of genetic information from the hexaploid.

In two instances, *Bowers & Wofford* (VDB) and *Raven 18704* (MO), the hybrids were collected along with *L. microcarpa* and *L. repens*; *L. palustris* was not observed locally. Although these plants, which have aborted ovaries, might have been considered as hybrids between *L. microcarpa* and *L. repens* on this basis, detailed examination of their flowers and leaves reveals that they are *L. microcarpa* × *L. palustris*.

Ludwigia polycarpa ($n = 16$) × **L. palustris** ($n = 8$; sect. *Dantia*). U.S.A. KENTUCKY: Ballard Co., at intersection of Kelley Branch Creek Rd. and KY 473, *Athey 1158* (NCU, NY, VDB). OHIO: Erie Co., in bottom of South Quarry at N edge of town on Kelley's Isl., *Stuckey 7400* (PH).

Notes. Although one of the putative parents, *L. polycarpa*, has not been recorded from Ballard Co. in extreme southwest Kentucky, it was collected from adjacent Massac Co., Illinois. *Ludwigia palustris* is common in Ballard Co., Kentucky. Plants of *Athey 1158* are comparable to artificial hybrids between these species synthesized in an experimental greenhouse.

In summary, geographical isolation and self-pollination are the primary factors limiting natural hybridization in species of sect. *Microcarpium*. For example, *L. microcarpa*, an extreme selfer, has been hybridized successfully with most species of sect. *Microcarpium* in the greenhouse, and the resulting hybrids were vigorous. It grows sympatrically with many other species (Fig. 21), but natural hybrids have been found only with *L. simpsonii*, *L. curtissii*, and members of sect. *Dantia*.

In general, however, natural hybrids in sect. *Microcarpium* are frequently found wherever two species occur together. This is particularly so for plants in the tetraploid group (including the hexaploid *L. alata*; Fig. 21), which were often found intermixed with their putative parents.

Especially evident in *L. pilosa* and hybrid populations involving *L. sphaerocarpa* were the effects of backcrossing and introgression, which may provide the hybrid populations with increased evolutionary flexibility, thus enabling them to grow in areas where neither of the parents are found.

As outlined above, natural hybridization is not limited to species within sect. *Microcarpium*. At

least seven hybrid combinations, some of which occur commonly in nature, have been found between members of sect. *Microcarpium* and sect. *Dantia*. Most of these have also been synthesized in the experimental greenhouse. Most intersectional hybrids, however, do not set seed, although they are usually very vigorous and appear to compete well with their parents. Once established, these sterile hybrids may be able to persist in a given location due to their perennial habit. New colonies may be established vegetatively if entire parents or fragments are transported to suitable habitats, most likely by water.

Species Relationships and Evolution

Among the four diploid species, *L. microcarpa* is quite distinct from *L. linearis*, *L. linifolia*, and *L. stricta*. Plants of *L. microcarpa* are small herbs with spatulate or obovate-spatulate leaves, minute apetalous flowers from which the pollen is shed singly, and short, tiny capsules; whereas the other three species have linear leaves, somewhat showy flowers with four petals from which the pollen is shed in tetrads, and elongate capsules. They appear to be relatively closely related to one another.

All eight taxa in the tetraploid ($n = 16$) group, including *L. alata* ($n = 24$), are apetalous. Their leaves range from linear-lanceolate to lanceolate, elliptic, or oblanceolate. They differ from each other in a number of characters, including capsule morphology, seed-surface pattern, pubescence, and the way in which pollen is shed.

Although *L. alata* and *L. simpsonii* are both hexaploids ($n = 24$), morphological characters suggest that they are not closely related. *Ludwigia alata*, which has winged capsules, is most similar to *L. lanceolata* of the tetraploid group, although it can be distinguished by having pollen shed singly, seed surfaces consisting of columnar cells elongate transversely to the seed length (Fig. 3), and by being modally outcrossing. *Ludwigia lanceolata* sheds its pollen in tetrads, has isodiametric seed-surface cells (Fig. 1), and is autogamous.

By contrast, hexaploid *Ludwigia simpsonii* resembles octoploid *L. curtissii* ($n = 32$). These two species can be distinguished relatively consistently only by the size of their mature capsules, although even this character is not always reliable. Both exhibit a specialized type of capsular dehiscence unique in sect. *Microcarpium*. Moreover, *L. simpsonii* and *L. curtissii*, along with the diploid *L. microcarpa*, are the only species in the section with spatulate or obovate-spatulate cauline leaves. There appears to be a close relationship between *L. microcarpa* and the *L. curtissii* complex.

Results from the crossing program and chromosome analysis of the artificial and natural hybrids confirm these general observations and provide additional evidence for some of the evolutionary relationships discussed below.

Relationships among the diploid species (*L. linearis*, *L. linifolia*, *L. stricta*, *L. microcarpa*)

The presence of a quadrivalent during meiosis of reciprocal hybrids of *L. linearis* × *L. linifolia* indicates that the genomes of the strains hybridized differ by a reciprocal translocation. Although reciprocal translocations are very frequent in the tribe Onagreae, they are rare in the remainder of the family; their presence here indicates that chromosomal repatterning has occurred between the strains hybridized. The artificially produced F_1 hybrids were very vigorous and set abundant seeds, although many of the F_2 plants were either weak or inviable. Presumably *L. stricta* will show a comparable degree of differentiation.

The hybrids between *L. microcarpa* and either *L. linearis* or *L. linifolia* showed very few (0–3) bivalents in meiosis. The bivalents sometimes appeared to be held together by matrix connections rather than by chiasmata; some of them were heteromorphic, and the chromosomes did not always line up in the equatorial plane. Taken together, these phenomena indicate that *L. microcarpa* has a diploid genome essentially different from that of either *L. linearis* or *L. linifolia*, and presumably *L. stricta* as well.

Relationships among the tetraploid taxa (*L. glandulosa* subsp. *glandulosa*, *L. glandulosa* subsp. *brachycarpa*, *L. lanceolata*, *L. pilosa*, *L. polycarpa*, *L. ravenii*, *L. sphaerocarpa*, and *L. suffruticosa*)

This is a group of eight diverse and morphologically well-delimited taxa. Artificial hybridization between any two species nearly always resulted in vigorously growing individuals with nearly complete chromosome pairing, high levels of stainable pollen, and abundant seeds. Many vigorous F_2 plants were raised that exhibited various degrees of intermediacy between the parents. This group is interpreted as representing an assemblage of interfertile tetraploid species that have two genomes in

common and thus represents a homogamic complex.

ORIGIN OF THE TWO GENOMES IN THE TETRAPLOID SPECIES

Most of the tetraploid species have been crossed with each of the three diploid species included in this study in order to assess whether one or more of the extant diploid species has been involved in their formation. *Ludwigia linearis* and *L. linifolia* were shown to share a similar genome that has undergone some chromosomal repatterning. Hybrids between either of these diploid species and any tetraploid species produced 3–7 bivalents in meiosis. Hybrids between *L. microcarpa* and the tetraploid species showed 1–6 bivalents in meiosis. Two lines of evidence suggest that the chromosomal pairing in these hybrids is the result of pairing of chromosomes between the diploid and the tetraploid rather than pairing between the two genomes present in the tetraploid. First, hybrids between *L. linifolia* (♀; $n = 8$) and *L. lanceolata* (♂; $n = 16$) were themselves tetraploid, modally forming 8 bivalents and 16 univalents in meiosis. This tetraploid chromosome number apparently resulted from the union of an unreduced egg from the diploid parent with a normal sperm nucleus from the tetraploid. This unexpected result strongly suggests that the 8 bivalents observed represent paired chromosomes from the duplicated *L. linifolia* genomes, while the 16 univalents are the chromosomes from the two genomes of the tetraploid parent, which are sufficiently different to remain unpaired. Pairing between chromosomes of *L. linifolia* and those of the tetraploids is evidently precluded by preferential pairing between the two sets of chromosomes derived from *L. linifolia*.

Second, when *L. glandulosa* ($n = 16$) was crossed with *L. alternifolia* ($n = 8$), a less closely related species belonging to sect. *Ludwigia*, the resulting hybrids exhibited 0–1 bivalent(s) in meiosis; the one bivalent that was occasionally observed was only loosely associated. This lack of pairing also suggests that the tetraploid species are alloploids with two unlike genomes.

As noted earlier, two distinct genomes appear to be represented among the diploid species; one shared by *L. linearis*, *L. linifolia*, and probably *L. stricta*, and another found in *L. microcarpa*.

Hybridization between any of these species and the tetraploid taxa consistently results in F_1 offspring that show only about 2–7 bivalents, some of them heteromorphic, in meiosis. This strongly suggests that neither of the two genomes present in the tetraploids was derived from an existing diploid.

ORIGIN OF GENOMES IN THE HEXAPLOID *LUDWIGIA ALATA*

Nearly all of the reciprocal hybrids between the hexaploid *L. alata* and the tetraploid species exhibited a modal meiotic configuration of 16 bivalents and 8 univalents. This suggests that they share two genomes: the 16 chromosomes of the tetraploid species pair with their homologues present in the genome of the hexaploid species, while the additional eight chromosomes in *L. alata* remain unpaired and appear to represent a third genome present in *L. alata*.

Ludwigia alata was also crossed with the diploid species in an attempt to assess whether this "third genome" is in fact homologous to one of those present in the existent diploid taxa. Hybrids between *L. alata* and either *L. linearis* or *L. linifolia* typically exhibit a configuration of 1–2 trivalents and 4–6 bivalents with the rest of the chromosomes univalents. Again, heteromorphic pairs are common here. These results are similar to those observed in hybrids between *L. linearis* and *L. linifolia* and the tetraploid taxa. Since the two genomes of the tetraploid species appear to be present in *L. alata*, it is presumed that the bivalents are formed between chromosomes derived from the parental taxa. By contrast, meiosis in hybrids between *L. microcarpa* and *L. alata* shows a high degree of chromosome pairing, with a modal configuration of one quadrivalent, 7–10 bivalents, and a corresponding number of univalents. The maximum amount of pairing seen was 1II + 10I or 1III + 10II + 8I. These chromosome associations involve bivalents of normal appearance, either rings or rods, a situation that contrasts with the sorts of loosely associated bivalents characteristic of hybrids between *L. microcarpa* and the tetraploid taxa. The higher level of chromosome pairing (more than eight bivalents) and the normal appearance of the pairs suggest that the hexaploid *L. alata* may have been derived following hybridization between *L. microcarpa* and one of the tetraploids. Indeed *L. alata* is similar to *L. microcarpa* in that (1) its seed surface consists of columnar cells transversely elongate to the seed length, and (2) its pollen grains are shed singly. These characters are rarely found among members of the tetraploid species group, with which *L. alata* shares two genomes.

ORIGIN OF GENOMES IN THE *LUDWIGIA CURTISSII* COMPLEX

The *L. curtissii* complex consists of *L. simpsonii* ($n = 24$) and *L. curtissii* ($n = 32$). Hybrids between the two species consistently reveal 24 bivalents and 8 univalents in meiosis. This indicates that chromosomes of the three genomes in *L. simpsonii* pair with homologues in *L. curtissii*, leaving the eight additional chromosomes in this species unpaired. Naturally occurring intersectional hybrids between *L. repens* ($n = 24$; sect. *Dantia*) and *L. simpsonii* produce a meiotic configuration of 48 univalents or 46 univalents and two loosely associated chromosomes. This strongly suggests that the three genomes in *L. simpsonii* are distinct from each other and not homologous with any of the genomes present in *L. repens*.

For ease of discussion, letters will be used to designate distinct, nonhomologous genomes. Since the five genomes present in *Ludwigia* sect. *Dantia* have been designated as A, B, C, D, and E (Schmidt, 1967), the genomic formula GGHHII will be used for *L. simpsonii* and FFGGHHII for *L. curtissii*.

The natural hybrid *L. microcarpa* × *L. simpsonii* has a meiotic configuration of 8 bivalents and 16 univalents. The eight chromosomes of *L. microcarpa* thus have 8 homologues in *L. simpsonii*, and its genome is designated as GG.

A configuration of 8 bivalents and 24 univalents is expected in meiotic cells of the hybrid *L. curtissii* (FFGGHHII) × *L. microcarpa* (GG). In a natural hybrid of this combination, the single analyzable cell did indeed show this configuration. In an artificial hybrid, however, more than 8 bivalents have been observed: five cells exhibited 14–15 bivalents and 12–10 univalents. Some intergenomic interaction must have led to the observed configurations.

In the meiosis of *L. curtissii* (FFGGHHII) × *L. linifolia* ($n = 8$), a modal configuration of 8 bivalents and 24 univalents was observed. The genome common to *L. linearis* and *L. linifolia* is designated FF, since it differs from that present in *L. microcarpa* (GG). As in the case of *L. curtissii* × *L. microcarpa*, however, a few meiotic cells in *L. curtissii* × *L. linifolia* exhibited more than 8 bivalents; up to 12 have been observed.

Figure 23 summarizes the chromosomal homologies in the *L. curtissii* complex. The hexaploid *L. simpsonii* appears to have been derived from three different diploid lines. Morphological as well as chromosome pairing data indicate that the diploid *L. microcarpa* has been involved in the formation of *L. simpsonii*. Based on morphology, it seems unlikely that a member of the *L. linifolia*/*L. linearis* lineage could have been a parent of *L. simpsonii*. Nevertheless, it will be necessary to examine meiosis in the artificial hybrid between *L. simpsonii* and *L. linearis* or *L. linifolia*. Although plants of *L. simpsonii* × *L. linearis* were available, my attempts to study meiosis in them were unsuccessful. The octoploid *L. curtissii* was probably derived following hybridization between a diploid similar to *L. linearis* or *L. linifolia* with the hexaploid *L. simpsonii*, based on morphological data and crossing relationships.

To study the genetic relationships among plants from the tetraploid group (including the hexaploid *L. alata*) and the *L. curtissii* complex, 11 artificial hybrids were produced, 5 of which have been studied cytologically. The number of bivalents observed ranged from 4 to 13; a few trivalents and quadrivalents were also frequently seen (Table 15). Chromosome associations between the polyploids of the tetraploid group and the three diploid species, on the one hand, and those observed between the *L. curtissii* group and the diploid species, on the other hand, are consistent with these results.

Following the differentiation of diploid species of sect. *Microcarpium*, some have evidently become extinct. Natural hybridization between the diploid lineages followed by polyploidy has played a major role in the evolution of this group. Postzygotic genetic barriers do not exist between most of the extant species in the section. Rather, the major limiting factor to natural hybridization appears to be the modally autogamous breeding system of most species. Geographical isolation is important only with respect to *L. polycarpa*, which is distributed well to the north of nearly all the other taxa.

Natural hybridization is prevalent within sect. *Microcarpium*, and hybrids often occur in more or less undisturbed habitats where the parental taxa also grow. All interploid hybrids are sterile except for crosses between *L. alata* ($n = 24$) and the tetraploid species ($n = 16$), and crosses between *L. curtissii* ($n = 32$) and *L. simpsonii* ($n = 24$). Even sterile hybrids can persist and form large colonies, at least locally, and compete effectively with their parents, because of strong vegetative reproduction by means of stolons. Natural hybrids are especially common among members of the tetraploid group (including *L. alata*) and are nearly always vigorous and fertile.

Particularly complex is the pattern of variation in the tetraploid *L. sphaerocarpa*, which is apparently comprised largely of widespread stabilized

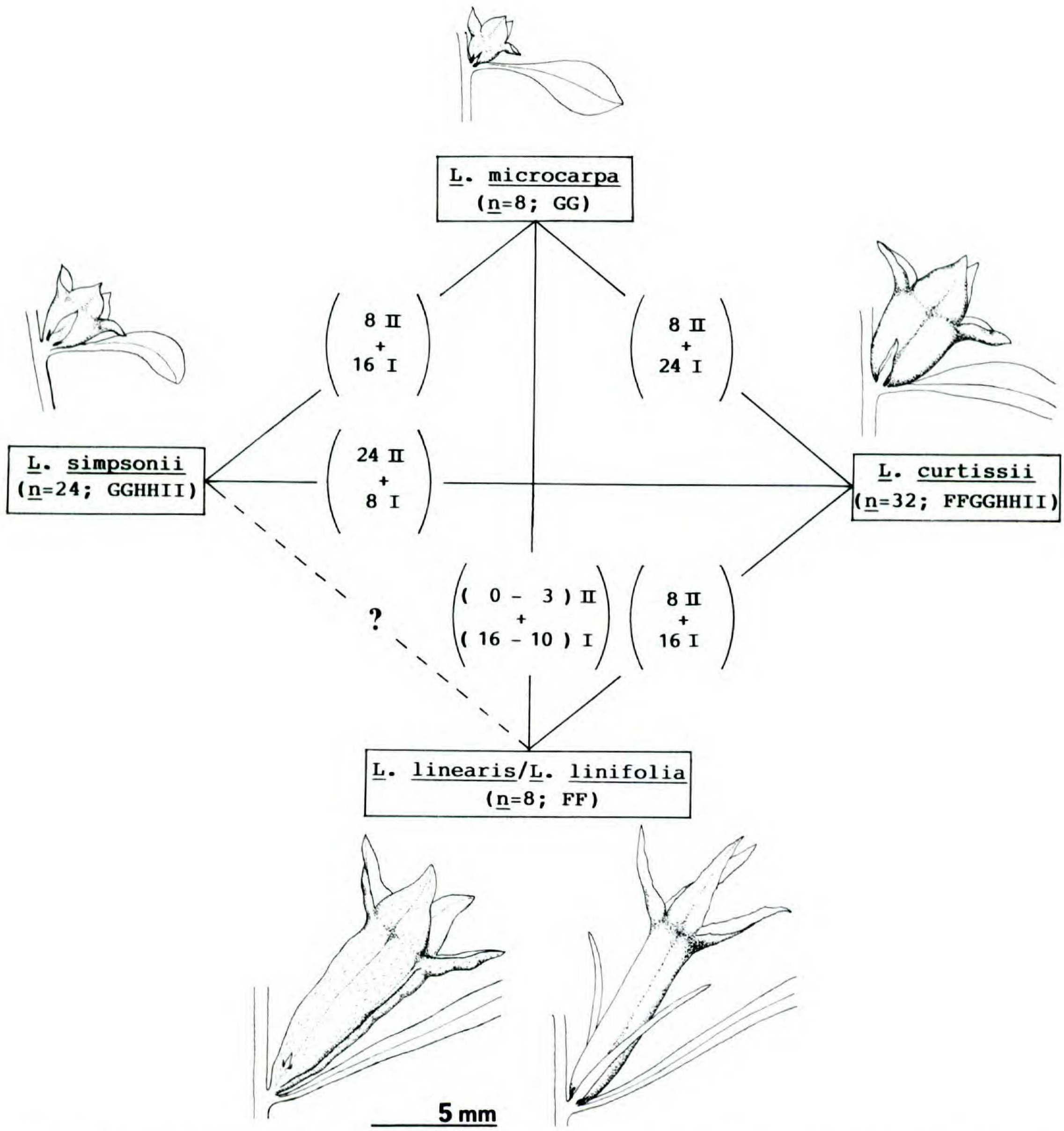

FIGURE 23. Chromosomal homologies in the *Ludwigia curtissii* complex. Dotted line indicates artificial hybrids were obtained, but meiotic analysis was not successful. Illustrations of the plants are drawn to scale.

hybrid populations that exhibit a combination of characters distinguishing them from other taxa. As in the evolution of *Epilobium* in New Zealand (Raven & Raven, 1976), recombination of genetic information from somewhat differentiated populations followed by maintenance of well-adapted genetic strains by a combination of autogamy and vegetative reproduction appears to have played a central role in the evolution of the polyploid members of *Ludwigia* sect. *Microcarpium*.

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